EVALUATION OF HAIR GROWTH POTENTIAL OF KOMBUCHA EXTRACTS TOPICALLY IN MICE

Prepared by

Mohammed Ayad Salih

Supervised by

Dr. Manal Najdawi

Co-Supervised by

Dr. Qais Jarrar

A Thesis

Submitted to Faculty of Pharmacy as a Partial Fulfillment of the Requirements for the Master's Degree in Pharmaceutical Sciences.

Faculty of Pharmacy

Isra University

August, 2020

جامعة الاسراء

نموذج التفويض

أنا محمد اياد صالح حمد، أفوض جامعة الاسراء بتزويد نسخ من رسالتي / أطروحتي للمكتبات أو المؤسسات أو الهيئات أو الأشخاص عند طلبهم حسب التعليمات النافذة في الجامعة.

التوقيع:

التاريخ: ٢٠٢٠/٦/١٩

Al-Isra University

Authorization Form

I'm Mohammed Ayad Salih Hamad, authorize Al-Isra University to supply copies of my Thesis to libraries or establishments or individuals on request, according to Al-Isra University regulations.

Signature:

Date: 6/9/2020

COMMITTEE DECISION

This Thesis (Evaluation of Hair Growth Potential of Kombucha Extracts Topically in Mice) was successfully defended and approved on 27/8/2020

Examination Committee	**Signature**
Dr. Manal Najdawi , (Supervisor)	----------
Dr. Qais jarrar . (Co-Supervisor)	----------
Dr. Amjad abu irmeileh. (Member)	----------
Dr. Bassam Amro (Member) (Jordan University)	----------

DEDICATION

Making this thesis come alive was one of my biggest dream in life, even when I was experiencing hardship and depression due sudden detours in my life, I still managed to persevere and complete my work. It wasn't easy but somehow imade it through. I'd like to thank almighty God for giving me the patience and determination to make it happen. I've finally made it.

For my father, Ayad Salih Al-buoraedi, you work tirelessly all your life so I wouldn't go without. I've never said this to you but I'm indebted to you for everything you've done. I want you to know that your support mattered immensely to me. Your enthusiasm for my work release encouraged me to make it happen. I hope I've made you proud and that you're pleased with how far I've come despite the obstacles I've faced and criticisms levelled against me.

For my mother, whose words were a remedy and who never stopped believing in me, always prayed and wished the best for me. I love you always and pray that I've made you proud too.

For my elder sister and her family, who helped me overcome the challenges I experienced writing this thesis. You supported me immensely, you are my backbone. You are always there for me. You encouraged me with your words of wisdom when I felt hopeless about myself and the world. Thank you for always being there for me.

For my little brother Ahmed and my sister Farah , thank you for support me.

AKNOWLEDGEMENT

I’d like to express my honest thankfulness to, Dr. Manal Najdawi and Dr. Qaiss Jarrar, my supervisors for the continuous support of my master's degree research, for theme motivation, patience, enthusiasm, and immense information. them leadership assisted me in all the time of research work and writing of the thesis. I couldn’t have imagined having better supervisors and mentors for my research.

Beside my supervisors, I’d like to thank the rest of my thesis committee, for their encouragement and insightful comments. I’d also like to thank Isra University for giving me the opportunity for doing this research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION OR SYMBOLS

Abbreviation or symbols	Definition
CAM	Complementary associated medicine
AGA	Androgenetic alopecia
SCOBY	Symbiotic culture of bacteria and yeast
GLcUA	Glucuronic acid
MSM	Methylsulfonylmethane
Vit BH	Biotin
FDA	Food and Drug Administration
SarcKATP	Sarcolemmal ATP-sensitive potassium
DHT	Dihydrotestosterone
SADBE	Squaric acid dibutylester
DPCP	Diphenylcyclopropenone
DNCB	Dinitrochlorobenzene
EGC	Epigallocatechin
EC	Epicatechin
DPPH	2,2-diphenyl-picrylhydrazyl
DSL	D-saccharic acid-1,4-lactone
CHD	Coronary heart disease
HDL	High-density lipoprotein
CVD	Cardiovascular disease
Vit C	L-ascorbic acid
ROS	Reactive O species
DMSO	Dimethylsulfoxide
IACUC	Institutional Animal Care and Use Committee
SEM	Standard error of the mean
Vit B1	Thiamine
Vit B6	Pyrodixine
Vit B2	Riboflavin
Vit B12	Cobalamin
Zn	Zinc

RNA	Ribonucleic Acid
DNA	Deoxyribonucleic Acid
Fe	Iron
AHA	Alpha hydroxyl acid
cAMP	Cyclic adenosine monophosphate
TGF	Transforming growth factor
EGCG	Epigallocatechin gallate
GnRH	Gonadotropin-releasing hormone
FMAP	Federal Medical Assistance Percentages
PGAs	Prostaglandin analogs
ORS	Outer Root Sheath
6-BA	6-benzyl-aminopurine
PDA	Pentadecanoic acid

Evaluation of Hair growth Potential of Kombucha Extracts Topically in Mice

Prepared by

Mohammed Ayad Salih

Supervised by

Dr. Manal Najdawi

Co-Supervisor

Dr. Qais Jarrar

Abstract

Consuming functional foods and drinks such as kombucha tea is believed to give many beneficial effects for promoting immunity and preventing cancer. This study was carried out to investigate hair growth potential of kombucha black tea and chamomile tea with and without methylsulfonylmethane (MSM) *in vivo* using Balb/c mice. The mice were divided into eight groups; minoxidil 2% as the positive control group, distilled water as the negative control group, black tea and chamomile tea as the control group, and the test groups which include kombucha fermented in black tea with and without MSM, kombucha fermented in chamomile tea with and without MSM respectively. The preparations were applied topically to the mice and observed for 30 days. Hair growth initiation time was observed for all groups, the results showed that treatment with kombucha/black tea with MSM reduced the time for hair growth initiation by 7 days. Hair density was assessed and the results indicated that there was a significant enhancement in hair density approximately 70% (P<0.0001) after applying kombucha/ black tea with MSM. Hair length were measured in two intervals the 15^{th} and 30^{th} day; the results showed a significant elongation of hair with p-value ≤ 0.0001 for the mice group that was treated with kombucha black tea with MSM.

From the current study, we can conclude that kombucha fermented in black tea with MSM can be considered a natural and effective alternative therapy for hair loss, in addition to a cost-effective treatment with fewer side effects compared to synthetic drugs.

Chapter one

INTRODUCTION

1. Introduction

It is believed that hair is our crowning glory playing a key role in determining who we are in most cases. However, this glory is sometimes fraught with difficulties and problems that may have an effect on our souls as well as our appearance. One of the most well-known problems is alopecia (or baldness), a dermatological disorder that results in partial or complete hair loss. Of the world's population between 0.2% and 2% are estimated to suffer from alopecia. Considerable efforts have been made since time immemorial to help find a lasting solution fixing this stressful problem. For example, over only the past few years millions of dollars have been spent on producing thousands of synthetic pharmaceutical products. But a number of constraints served as barriers towards the eradication or mitigation of this annoying problem, such as; lack of interest in inspecting the mechanisms of hair growth or loss, and poorly-targeted drug therapy. Over the recent several year's thousands of products have demanded to help hair regrowth. With the exclusion of minoxidil and finasteride, in which both are slightly effective at arresting the rate of hair loss and cannot stop it completely. In addition, they have disagreeable side effects, with finasteride being inappropriate for women and induce erectile dysfunction in men (Tosti and Duque-Estrada, 2009).

Many pharmaceutical agents are available in the market to manage hair loss but because of their limited efficacy, associated adverse effects, tolerance, and lack of proper information. It seems appropriate to identify alternative natural agents for the management of hair loss; herbal products have been widespread in hair care products and accepted in cosmetic industry, with numerous plant extracts have been inspected with regard to promoting hair growth activity, hardly few showed enormous potential effect on hair regeneration. The present research focuses on the assessment of a recently popular natural microbiome kombucha extract from black and chamomile tea as an alternative natural agent for the management of hair loss since many people frequently turn to complementary associated medicine (CAM) and unconventional medicine in trying to find safe, natural and effective therapy to restore their hair (Jayabalan et al., 2014).

1.1. Hair growth cycle

The natural output of the process of cellular differentiation and cellular proliferation inside follicles is hair. Particular dermal fibroblasts substitute on bipotential epithelial stem cells produce hair follicles. Hair follicles go through a cycle of 3 stages; anagen, catagen, telogen,

and exogen {growth (active) phase, degenerative (transition) phase, resting and shedding phase} respectively. The lifetime of each phase is different; the anagen phase ranges between 2-5 years, at any given time around 85%–90% of the scalp hair is in this phase; the catagen phase ranges between a few days to a few weeks, and 1% of scalp hair stays in this phase; the telogen phase takes around 1-4 months, in which 10-15% of the head's hair is in this phase (Wood and Price, 1999). This cycle is an extremely intricate process and involves apoptosis (programmed cell death), pigmentation, stem cell augmentation, epithelial-mesenchymal interactions, pattern formation, cell differentiation, and cell organ growth cycles. Any change occurring to the lifetime of any of the phases can have a visible and direct effect on the hair growth, for instance; if anagen comes into a premature end and catagen begins too early, the ultimate result will be alopecia or effluvium. The affected area of the skin will then have an immense number of catagen or/ and telogen on the scalp. This is exactly what happens, for instance, because of drug-induced damage to the increasing anagen hair bulb, for example in drug-induced telogen effluvium or when inflammatory cells attack the anagen hair bulb in alopecia areata. Thus, making changes or modifications to the hair follicle cycle is quite challenging.

Figure 1. Hair growth cycle.

(Barrera, 2005).

In hair loss therapy the strategies adopted in reducing hair loss are; suppresing the catagen phase to prolong anagen phase, and repressing the exogen (shedding) phase (Barrera, 2005).

1.2. Alopecia and hair loss

In normal circumstances, the head sheds between 50-120 hairs (depending on gender), however after the resting phase, the follicles will grow back. Indeed, this cycle doesn't occur chaotically since each hair is programmed genetically to a certain cycle of growth, stabilization, aging, and shedding (Messenger and Rundegren, 2004). When the hair loss surpasses the growth phase, or when the growth is not strong enough the hair problems will arise, that is to say; the anagen period is lessened. On average, it is normal to shed up to 100 hairs a day, but exceeding this number is considered a dermatological condition (effluvium) (Levy and Emer, 2013). A very common pathological condition affecting 50% of men over 50 years of age, and 30% of men under 30 years is what is called androgenetic alopecia (AGA), affects not only men but also women (despite having a different phenotype and the mildness of clinical signs). In men, this condition is also known as male-pattern alopecia. Hair is lost in a well-defined pattern, beginning above both temples. In women, typically thinning occurs at the top of the head. Over time, the hairline recedes to form a characteristic "M" shape. Androgenetic alopecia can be given rise to a variety of factors restricted to the actions of hormones, including some ovarian cysts, taking high androgen index birth control pills, pregnancy, and menopause (Carmina and Lobo, 2003).

The classification of alopecia is categorized as follows: Female pattern alopecia, Male pattern alopecia, Alopecia areata, Alopecia totalis and Alopecia Universalis. Hair loss also can be sorted based on the leading factors:

Anagen effluvium: due to certain medications like chemotherapy, some medications intended for high blood pressure or vitamin A overdose.

Telogen effluvium: as a result of a growing number of follicles getting into the resting phase that is caused by stress (emotional or physical), hormones, and thyroid dysfunction (Kaushik, Gupta and Yadav, 2011).

1.3. Current strategies for hair growth and regeneration

There are several ways through which medicine might promote hair growth, that is classified based on their mechanism of action which includes: Potassium channel openers (e.g minoxidil, pinacidil, and diazoxide) (Vexiau *et al.*, 2002), Antiandrogen (e.g cyroterone acetate), 5-α-reductase inhibitors (e.g. finasteride, dutasteride, and episteride), Nutrients and minerals (e.g. Vitamin C, B, and E; zinc, amino acid, and essential fatty acid) and Miscellaneous (e.g. eflornithine, all-trans-retinoic acid, and other new hair growth-promoting agents) (Semalty *et al.*, 2011).

Therefore, modifying the hair cycle, enlargement of the hair fiber, prolongation of the anagen phase, or shortening the telogen phase, or a combination of some of them, leads to hair regeneration or re-growth using the above-mentioned agents.

Surgical treatments, such as micro-grafts and mini-grafts have made considerable progress in treating male pattern and female androgenic alopecia (Barrera, 2005).

1.4. Kombucha

Kombucha is a traditional fermented beverage originated in China, obtained through the fermentation of sugared black or green tea with a symbiotic culture of bacteria and yeast (SCOBY) (Jayabalan *et al.*, 2014). The beverage anecdotally is known to possess many therapeutic benefits claims which include relieving the symptom of arthritis, wound healing, improving gut health, fighting cancer, reducing hair loss, relieving hemorrhoids' symptoms, and promoting mental and liver health (Amarasinghe, Weerakkody and Waisundara, 2018). The microbe populations in kombucha vary; the yeast part usually includes *Zygosaccharomyces bailli*, in conjunction with different species; the bacterial element includes Acetobacter to oxidize the alcohol produced from the yeast to acetic acid, and different acids (Jarrell, Cal and Bennett, 2000).

1.4.1. Chemical component of kombucha and their beneficial effects

Kombucha chemical assesses included a variety of compounds (Figure 2), such as; organic acids, mainly gluconic, acetic and glucuronic (GlcUA) acid, although pyruvic, succinic, malic, oxalic, L-lactic, malonic, citric, usnic acids and tartaric may also be identified;

hydrolytic enzymes, water-soluble vitamins (C, B12, B6, B2, B1), sugars (fructose, sucrose, and glucose), carbon dioxide, ethanol, proteins, acetic acid bacteria, and lactic acid bacteria, biogenic amines, lipids, amino acids, pigments, purines, polyphenols, (copper, nickel, plumb, zinc, chromium, cobalt, iron, manganese and cadmium), anions (bromide, chloride, fluoride, phosphate, nitrate, sulfate, and iodide), D-saccharic acid-1,4-lactone (DSL), and metabolic crops of bacteria and yeasts (Jayabalan *et al.*, 2014). The quantity and presence of the chemical components vary, and this is according to the sucrose content, type of tea used, fermentation temperature and time, the analysis methods used for quantification, and the microorganisms of the symbiotic culture used for fermentation of kombucha.

Some metabolic products of SCOBY like acetic acid and other organic acids, posse's antibacterial activity and prevent contamination of the drink by pathogenic bacteria (Watawana *et al.*, 2015).

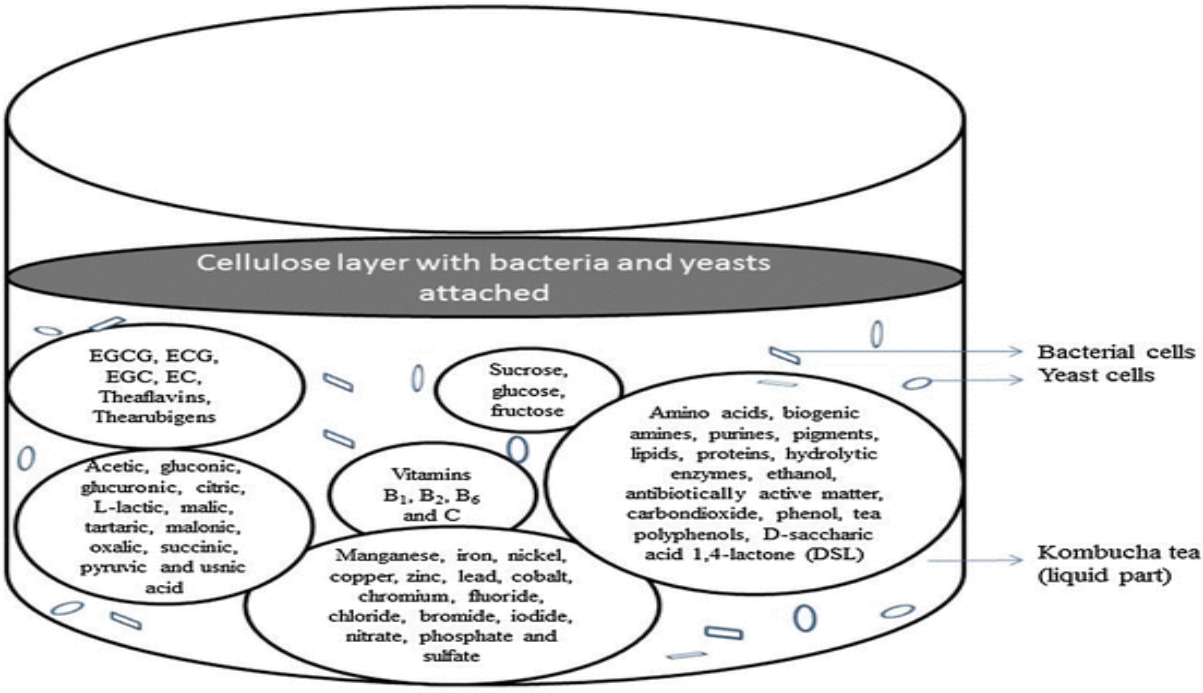

Figure 2. bioactive components of kombucha

(Watawana *et al.*, 2015).

Table (1). Beneficial metabolites that are produced in kombucha. (Amarasinghe, Weerakkody and Waisundara, 2018).

Reported effects of kombucha from tea drinkers	
1. Detoxify the blood	2. Prevent/heal bladder infection and reduce kidney calcification
3. Reduce cholesterol level	4. Stimulate glandular systems
5. Reduce atherosclerosis by regeneration of cell walls	6. Protect against diabetes
7. Reduce blood pressure	8. Increase body resistance to cancer
9. Reduce inflammatory problems	10. Have an antibiotic effect against bacteria, viruses, and yeasts
11. Alleviate arthritis, rheumatism, and gout symptoms	12. Enhance the immune system and stimulate interferon production
13. Promote liver functions	14. Relieve bronchitis and asthma
15. Normalize intestinal activity, balance intestinal flora and cure hemorrhoids	16. Reduce menstrual disorders and menopausal hot flashes
17. Reduce obesity and regulate appetite	18. Improve hair, skin, and nail health
19. Reduce an alcoholic's craving for alcohol	20. Improve eyesight
21. Reduce stress and nervous disturbances, and insomnia	22. Counteract aging
23. Relieve headaches	24. Enhance general metabolism

1.5.Aims and objectives:

Evaluate, for the first time, the efficacy of the bioactive components of kombucha produced with black and chamomile tea, using balb/c mice as an *in vivo* model topically through the comparative examination with 2% minoxidil as a control treatment on:

Hair growth initiation time, Hair density and Hair length.

Investigate tolerability of the topical application of kombucha as acidic preparation on the skin of denuded mice.

Assess the effect of the delivery of the bioactive component of kombucha extract by incorporating MSM as a penetration enhancer.

Explore the effect of blank chamomile and black tea using the same parameter mentioned above on the stimulation of hair growth.

Chapter two

PREVIOUS STUDIES

2. Previous studies

2.1. Anti-androgen

Spironolactone a synthetic steroid is the most frequently prescribed antiandrogen to treat female pattern alopecia. It slows down the production of androgen, in addition, lower the effects of DHT by blocking competitively the binding to its receptor, thus preventing miniaturization of the hair follicle (Levy and Emer, 2013).

Cyproterone is a synthetic steroid act by directly block androgen receptor and reduce testosterone levels by suppressing gonadotropin-releasing hormone (GnRH) (Coneac, Muresan and Orasan, 2014). A study comparing cyproterone acetate with 2% minoxidil showed that cyproterone acetate was more successful in reducing hair loss in women with signs of hyperandrogenism (Vexiau *et al.*, 2002).

ketoconazole and imidazole derivative showed to be effective in the treatment of AGA, stem from its effect on reducing the level of DHT by inhibiting the synthesis of testosterone, in addition, it has anti-inflammatory activity by blocking 5-lipooxygenase enzyme (Levy and Emer, 2013) (Aldhalimi, Hadi and Ghafil, 2014).

Flutamide is an antiandrogen that selectively binds to the androgen receptor. A study by Carmina showed that 250mg flutamide daily improved the growth of hair in Federal Medical Assistance Percentages (FMAP) compared to 5mg finasteride and 50mg cyproterone acetate daily (Carmina and Lobo, 2003). Flutamide is an alternative treatment choice for patients with a normal level of androgen as a proof in a treatment given for females with 250mg compared with spironolactone, but because of its liver dysfunction that is dose-dependent should be given with caution (Yazdabadi and Sinclair, 2011).

2.2. Five- alpha reductase inhibitors

Finasteride is a 4-aza steroid acting as an inhibitor to type II 5-alpha reductase (enzyme) which blocks the conversion of testosterone into the dihydrotestosterone (DHT) form, responsible for hair miniaturization (Zaccheo, Giudici and Di Salle, 1998).

It was originated to be used for the treatment of enlarged prostate or benign hyperplasia. Apparently, blocking dihydrotestosterone (DHT) action induces hair growth by making it more pigmented, thicker, and stronger. In a research conducted on macaque monkeys, targeting baldness in adult male stump tail for the determination of the effects on scalp hair growth,

finasteride, when orally taken alone at 0.5 mg/day, or combined with topical 2% minoxidil for 20 weeks, a 1-day dose survey showed that both 2.0 and 0.5 mg doses similarly reduced serum dihydrotestosterone in male stump tails (Diani *et al.*, 1992). Another research found that finasteride noticeably regrew hair in about 40% of balding men (Rushton *et al.*, 2002). Finasteride is probably not effective in males over 60 years (Wood and Price, 1999). Although Dihydrotestosterone (DHT) is necessary for males to have a normal sexual life, around 3% of the sample has problems as side effects regarding sexual performance such as diminution in sperm counts, impotence, erectile dysfunction or loss of libido. Nevertheless, this steroid isn't approved for females (because even small concentrations cause hypospadias) and has not shown any effectiveness in the temple receding hairline.

Dutasteride showed to be a more potent inhibitor for reductase enzyme since it has an inhibition effect on 5α-reductase for both types I and II, showing enhanced hair growth in men with hair loss, faster and immensely than the selective type II inhibitor finasteride (Olsen *et al.*, 2006). Dutasteride 0.5mg daily reduces 90% 0f DHT in the blood in comparison to 1mg which showed to be 70% (Eun *et al.*, 2010).

2.3. Potassium channel openers

Minoxidil tablets were used as an antihypertensive, some patients treated with minoxidil showed hypertrichosis (excessive hair growth) as a side effect. Also, the Robertson et.al showed that hair loss can be inhibited by applying the topical solution of minoxidil (the only drug approved by the FDA) to the scalp. Minoxidil oral tablets function as an antihypertensive and works as an opener of sarcolemmal KATP (Sarcolemmal ATP-sensitive potassium (sarcKATP) channels were proposed to play an important role in the cardioprotective effect) channels, by relaxing vascular smooth muscle by its sulfated metabolite action, minoxidil sulfate. The opening of potassium channels by minoxidil sulfate has a stimulatory effect on hair growth, but it's hard to establish that KATP channels are expressed in hair follicles (Robertson and Steinberg, 1990).Minoxidil's mission is to inhibit collagen synthesis, stimulate mitosis in epithelial cells, stimulate vascular endothelial growth factor and prostaglandin synthesis, and prolong the survival of epithelial cells in tissue culture. It is proposed that this medicine precludes follicles from turning into the following anagen stage, stimulates them back, makes them more productive, and restrain the aging of matrix cells. The optimum concentration of topical minoxidil solution to show effectiveness is 2% with a lifetime commitment for efficient therapy. It has to be applied to the balding region twice a day without

decreasing the dosage to once a day or discontinuing application because of regression to baldness in 3-6 weeks. Women with female pattern have noticed some improvement in psychological perceptions of hair loss (Buhl *et al.*, 1993).

Pinacidil, nicorandil, RP-49356, and P-1075 are K+-channel openers. Similar to minoxidil have a role in regulating the cycle of hair growth. Cell proliferation in dermal keratinocytes and cultured vibrissae is stimulated by the openers of the potassium channel. They provoke hypertrichosis in human beings (Goldberg, 1988) (Robertson and Steinberg, 1990). The effect of minoxidil, when topically applied, and three other potassium channel openers on scalp hair growth in balding macaques were tested by Buhl and showed that minoxidil, chromacalym, and P-1075 (an analog penasidel) stimulated hair growth over a treatment period of more than 20 weeks. however, RP-49356 didn't show any effectiveness. In 2–13% of patients, systemic pinacidil stimulates hypertrichosis (Buhl *et al.*, 1993).

Diazoxide another K+ channel opener which is reported to enhance the absorption of thymidine in a dose-dependent manner in 4 days of cultures of vibrissae follicles of mice (Messenger and Rundegren, 2004) (Harmon, Lutz and Ducote, 1993). Oral diazoxide showed to cause hypertrichosis in about 1% of adults and most hypoglycemic children and stimulated some scalp hairs in 25% of the patients suffering from baldness (Roenigk Jr, 1988).

2.4. Prostaglandin analogues

Recently, prostaglandin analogs (pgas) such as travoprost, latanoprost, and bimatoprost has been used topically in the treatment of ocular hypertension and glaucoma and unexpectedly found to activate the growth of the eyelashes resulting in darker, thicker and longer eyelashes, suggesting the probability of therapeutic use. It is suggested that the growth of the eyelashes attained through elongation of the anagen phase in the hair follicle (cohen, 2010). Despite latanoprost ability to induce eyelashes growth in a healthy individual but its role to reverse androgenic hair loss has not been supported by experiments, since the scalp hair follicle for androgenetic alopecia is different than the follicle in the normal eyelashes. Latanoprost topically with a high dose (500 µg/ml) for 5 months stimulated the growth of hair in the macaque model of androgenetic alopecia, by converting 5-10% of the vellus hairs to terminal hairs (uno et al., 2002). Another study by blume-peytavi showed that applying latanoprost 0.1% topically to the scalp for 24 weeks increased the density of hair significantly (blume-peytavi et al., 2012). However, faghihi assessed the topical application of latanoprost for the treatment of androgenic alopecia on both eyebrow and eyelashes and reported that in comparison to placebo

no significant effect was shown (faghihi, andalib and asilian, 2012). Therefore the use of pgas needs to be further evaluated to determine their efficacy on the scalp of androgenetic alopecia patients.

2.5 Natural products

Drugs made of herbs have been used and documented in old civilizations, like; (Chinese and Ayurvedic). It has also been reported that natural (herbal) alternatives proved by some authors to be more effective in hair growth than synthetic drugs. For potential clinical applications, several bioactive plant compounds have been tested. Table 2 demonstrates different natural hair growth products. Herbal drug researches were conducted to find an effective and safe alternative hair loss treatment. A major polyphenolic constituent of green tea, epigallocatechin-3-gallate has effective in the of androgenetic alopecia therapy through inhibiting 5-alpha reductase activity (Esfandiari and Kelly, 2005).

Table (2). Herbal drugs for hair growth promotion. (Esfandiari and Kelly, 2005).

Herbal drug	**Mechanism of preventing hair loss**
Grape seed	Contains proanthocyanidins, which are effective antioxidants and performance as a smooth muscle relaxant in blood vessels and capillaries, avoiding or offsetting damage to the hair follicle blood stream
Rosemary oil	Progresses blood flow to scalp . Cleansing the scalp and stimulating the hair root
Sage (Salvia officinalis)	Thickens hair shafts and helps dissolve sebum deposits . Improves blood flow to scalp
Emu oil	Inhibits 5-alpha reductase and thus lowers the DHT level in the scalp
Aloe vera	Its proteolytic enzymes slough off dead skin cells and open pores

	. Increases membrane fluidity and permeability and the outward flow of toxins and inward flow of Nutrients
Ginkgo biloba	Inhibits 5-alpha reductase activity . Protects small blood vessels and micro-capillaries against loss of tone and fragility
Bee pollen	Being rich in L-cysteine, it stimulates hair growth (since hair is 8% L-Cysteine)
Green tea	A potent inhibitor of 5-alpha reductase and thus lowers the DHT level in the scalp
Saw palmetto (Serenoa repens)	Blocks DHT production
Nettles (Urtica dioica)	Provides silica for hair growth . Improves blood flow to scalp
Hibiscus rosasinensis	Improves blood flow to scalp which leads to dense hair growth

Catechins of green/black tea and polyphenolic compounds (such as epigallocatechin-3-gallate) are the main phytoconstituents taking the responsibility for its hair growth. Adhirajan et al. assessed the effect of the petroleum ether extract of the flowers and leaves of *Hibiscus Rosasinensis* on the growth of hair through *in vitro* and *in vivo* ways (Adhirajan *et al.*, 2003). They found the leaf extract, to be more potent than the flower extract on hair growth. Adhirajan et al. observed in another research a combination of petroleum ether extract of *Citrullus colocynthis Schrad. (Cucurbitaceae), Eclipta alba Hassk. (Compositae), and Tridax procumbens Linn. (Compositae)* with different concentrations as an oil or cream. The ratio of *T. procumbens C. colocynthis and E. alba*, in 2:1:3 demonstrated some noticesble growth with 35% more anagen hair follicles compared with 20% with a standard drug (2% ethanolic solution of minoxidil) (Adhirajan, DIXIT and CHANDRAKASAN, 2001). Roy et al. tested the effect of ethanol extracts of *Cuscuta reflexa and C. colocynthis* and successive petroleum ether on hair growth in rats with albinism (R K Roy, Thakur and Dixit, 2007). Those extracts were integrated as a base of oleaginous ointment and topically applied on the albino rats' shaved skin. The time needed for the hair to grow and its growth cycle were recorded. Half of the hair growth starting time has been considerably decreased on treatment with the petroleum ether

extracts, when compared to the untreated control animals, and the time needed for the hair to grow was also significantly reduced. The therapy has proven successful since it brought a huge number of follicles (> 70%) to the anagen phase than standard minoxidil. The polyherbal formulation of *E. alba (Hassk.), C. colocynthis (Schrad.), and C. reflexa (Roxb.)* in another study was assessed and developed the same to promote hair growth. When compared to control animals, the hair growth starting time was noticeably decreased to 1/3 on treatment with the prepared formulation, (Ram Kumar Roy, Thakur and Dixit, 2007). The time needed for the hair to grow decreased by 32%. Uno et al. described a method called quantitative analysis of the hair growth cycle pursuant to the therapy along with minoxidil (2%) and prepared herbal formulations. The quantitative analysis exhibited a greater number of follicles in the anagen phase when compared to controls (Uno *et al.*, 2002). 45 plant extracts were tested by Rho et al. to see their effects since they have been prescribed to help stop hair loss in traditional oriental medicine for the identification of potential hair growth stimulants. There may be a hair growth-promoting potential by *asiasari radix* extract (Rho *et al.*, 2005). Rho et al. studied and showed, in another study, that *Sophora flavescens* promoted hair growth. The effectiveness of topical crude onion juice (*Alliumcepa L.*) was tested in patchy alopecia areata therapy. The patients were broken down into two groups; the 1st group (active: onion juice) comprising 23 patients (7 females and 16 males), the 2nd one (control: tap-water) comprising 15 patients (7 females and 8 males). Both the groups were recommended the treatment be applied twice a day for 2 months. In the active group after 2 weeks of therapy terminal coarse hairs started to grow. Hair re-growth was apparent in 17 patients (73.9%) after week 4, and it was noticeable in 20 patients (86.9%) at week 6; it was substantially higher in males (93.7%) when compared to females (71.4%). In the control group, hair re-growth was observed in only two patients (13%) after week 8 without any differences between males and females. *Illicium anisatum* extract enhances under the skin of mice. The research used an organ culture method to test this extract's follicle elongation effect, and B6C3HF1 vibrissae follicles of mice were cultured for 7 days at 31_C in a medium free of serum (Sakaguchi *et al.*, 2004). Follicles, which are treated with shikimic acid or water-soluble extracts of the fruits, roots, and leaves of *I. anisatum* considerably became longer than controls. Bisbenzylisoquinoline alkaloids were isolated from *Stephania cepharantha* and their proliferative activities of cultured hair cells from mice skin were assessed (Nakaoji *et al.*, 1997). *Cepharanoline, cepharanthine, berbamine, and isotetrandrine* showed considerable activities in the range of 0.01–0.1 mg/ml but did not show any active signs on cultured keratinocytes or fibroblasts from the mice skin. The cells stimulating the anagen stage, stem cells, were found in the bulge area of the outer root sheath (ORS). Different

botanical extracts' effect on the cultured human follicles growth was evaluated to detect agents that promote growth for the ORS cells. It was found that ORS cell growth was increased by *Laminaria angustata* extract. Moreover, the topical application of the extract also enhanced hair growth in the C3H mice shaved skin (Osawa *et al.*, 2004). The other natural herbal drugs tested for hair growth include *bahera (Terminalia bellirica),bhallataka (Semecarpus Anacardium), Brahmi (Bacopa monnieri), neem (Azadirachta indica), laljari (Geranium wallichianum), nagarmotha (Cyperus rotundus), methi (Trigonella foenumgraecum), capsicum and amla (Emblica Officinalis).* Some different herbal drugs have deserved to be granted various patents for the potential of hair growth, for example; *Fructus Mori, Angelicae Sinensis, Oleum ricin, Sesame nigrum, Flos carthami, Berberis vulgaris, Paenoiae Rubra, Ginkgo biloba, Pinellia ternata, Polygoni multiflora, Cacumen biota, Capsicum annum, and Zingiberis recens.*

2.5.1. Chamomile

The chamomile plant, which is scientifically referred to as *Chamaemelum nobile or Anthemis nobilis*, is native to Europe and North America. Its tiny yellow daisy-like flowers are the active part of the herb (A. Pirzad *et al.*, 2006). This plant has been used traditionally in making tea to treat and prevent many health issues including alopecia. Approximately 120 secondary metabolites have been identified in chamomile, including 28 terpenoids and 36 flavonoids (A Pirzad *et al.*, 2006). It's anti-inflammatory (*Chamazulene*), anti-microbial, and anti-irritant properties, chamomile helps in boosting hair growth. In addition, chamomile has a soothing and calming effect. It conditions the scalp, nourishes, and strengthens the hair follicles, invigorating the growth of thicker, fuller, and healthier hair fibers (Singh *et al.*, 2011).

2.5.2. Miscellaneous

Squaric acid dibutyl ester (SADBE), diphenylcyclopropenone (DPCP), and dinitrochlorobenzene (DNCB) are some of the topical sensitizers that have determined the hair re-growth in balding patients. In alopecia areata, the only non-specific irritant largely addressed for hair growth, Anthralin is topically applied as a 1% or 0.5% cream, for 20–45 mins, to affected regions, once a day; those who are able to stand the side effects are allowed for overnight application (Fiedler, 1992). Some recent hair agents promoting growth are cromakalim, infliximab, All-trans-retinoic acid [(tretinoin) (3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexenyl)-non-2,4,6,8 tetraenoic acid)], latanoprost, 6-benzyl-aminopurine (6-BA) and

pentadecanoic acid (PDA), RU5884, LY191704 (a benzoquinoline derivative), and etanercept (Kwon *et al.*, 2007).

2.5.3. Kombucha

Tea is one of the most common drinks and a necessary source for polyphenolic constituents. Polyphenols are found in tea from *camelia sinensis*; yet, due to the fermentation method used in black tea, the polyphenols types found are totally different. According to Scalbert et al., many *in vitro* studies proved that the polyphenols have metal chelating properties and strong antioxidant, therefore, they will cover DNA and proteins, cells and tissue structures - lipids, against ROS (reactive O species) (Scalbert, Johnson and Saltmarsh, 2005). There is a widespread belief that one of the oldest strategies for food preservation is fermentation. During this process, multiple biochemical changes take place and affect the properties of the final product, just like digestibility and bioactivity. This bioprocess has been recently used so as to extract and produce bioactive compounds from plants in the industries of beverage and food (Galati *et al.*, 2014). Kombucha beverage is attained by fermenting sugared black tea with a symbiotic bacteria and fungi. This fermented beverage anecdotally is known for having multiple therapeutic and prophylactic advantages. Jarrell et al., the microbic populations are various in kombucha; the bacterial part includes Acetobacter for the oxidization of alcohol produced from the yeast to acetic acid and different acids; and the yeast element usually includes *ZygoSaccharomyces bailii*, in conjunction with different species. This beverage's beneficial compounds include lactic acid, amino acids, gluconic acid, glucuronic acid, polyphenols (flavonoids), epigallocatechin gallates, and vitamin B12, B6, B2, B1 (Jarrell, Cal and Bennett, 2000).

Over the last few years, this live culture has been spotted as an active ingredient in skincare products because of its detoxifying and hydrating effect, with improvements in the elasticity of the skin, which combats the appearance of fine lines.

2.5.4. Other reported beneficial effects of kombucha

People who consumed this beverage stated that it has beneficial effects on human health. Aloulou et al. assessed the inhibiting effect of the α-amylase enzyme, which is secreted via intestinal epithelium and it is important for carbohydrate digestion, in diabetic rats (alloxan-induced), given as 5 mL/kg of black tea kombucha daily within 30 days (Aloulou *et al.*, 2012). The studies have shown that the rats which consumed kombucha got better inhibiting/

suppressing the effect of the α-amylase enzyme in plasma and pancreas, besides postprandial glucose compared to the ones of the rats that consumed black tea. Plasma enzymatic and pancreatic changes were evaluated, in addition to glucose metabolism disorders. In order for these enzymes to metabolize triacylglycerol into free fatty acids and monoacylglycerol, they act on it, and an abnormal increase may take place due to pancreatic damage (Sastre and Sabater, 2005). The rats which were given alloxan had more damage in pancreatic structure than the damage of those from the rats treated with kombucha or control group. Alloxan increases ROS (reactive oxygen species), which makes pancreatic cells intoxicated. An increase in plasmatic and pancreatic lipase concentration raises lipid absorption, contributing to low-density proteins and an increase of triacylglycerol. The enzymes of the group which was treated with black tea decreased, but this decrease was less than that of the group consuming kombucha. The group which was treated with kombucha showed a considerable decrease of plasmatic and pancreatic lipase. Setorkil and Ahangar Darabi, also scientifically proved in a study determining the protective effects of kombucha and milk thistle (silymarin) in rats suffering from liver damage arising from thioacetamide (toxin hepatic fibrosis) (Setorkil and Ahangar Darabi, 2013). In this study, 36 rats were grouped into 6 categories as follows; Group 1 was the control group, group 2 was the rats injected with thioacetamide, group 3 was the rats injected with thioacetamide and later treated with kombucha (50 mL within 3 weeks), group 4 was the rats treated with kombucha (50 mL within 3 weeks) and later injected with thioacetamide, group 5 was the rats injected with thioacetamide and later treated with silymarin (200 mg/kg within 3 weeks), and group 6 was the rats injected with thioacetamide and later treated with silymarin (400 mg/kg) and kombucha (50 mL/per rat) within 3 weeks. The group which was treated with silymarin showed a considerable decrease in the aforementioned parameters with exempt of bilirubin, and so was the group treated with kombucha tea and silymarin. Polyphenol component is responsible for the protective action in both foods, which provide the liver of protection against free radical formation which may cause liver damage and produce hepatocyte malfunction. Deghrigue et al., have studied antiproliferative characteristics of kombucha, prepared with green tea or black, which was under fermentation within 12 days, on two cell lines of human cancer (Hep- 2, epidermoid cell carcinoma and A549, lung cell carcinoma) (Deghrigue *et al.*, 2013). The cells were incubated in microtitre plates for 24 h, and kombucha, previously subject to centrifugal force, was added afterward (Leal *et al.*, 2018). For the purpose of determining IC50 (measurement unit for the effectiveness of a substance to inhibit a biological process at a value ≥ 50%), concentrations varied from 50–400 μg/mL. Kombucha drink which was elaborated with green tea showed more cytotoxic

effect. The inhibition of 50% has been obtained on the cell lines Hep-2 and A549 at concentrations ranging from 200 to 250 μg/mL. Conversely, kombucha drink which was elaborated with black tea had a moderate cytotoxic activity; it required greater concentrations to inhibit 50% of cellular growth, once compared to the green tea-based kombucha, 386 μg/mL, and there was an effect only on Hep-2 cell lines. For preparing kombucha infusions of oak leaves instead of black tea as a common substrate have been used by (Vázquez-Cabral *et al.*, 2017). They indicated a considerable decrease in the levels of pro-inflammatory cytokines IL-6 and TNFalpha. Also, oxidative stress was reduced by phytochemical compounds contained in the fermented drink. With respect to the increase in kombucha's antioxidant activities and phenolic compounds, Sun, Li, and Chen elaborated the drink with mixes in different ratios of wheatgrass juice and sweetened black tea (Sun, Li and Chen, 2015). The highest antioxidant activity was attained using a 1:1 (v/v) black tea decoction to wheatgrass juice ratio and 3 days of fermentation. This drink, under these conditions of processing, produced different kinds of complementary phenolic acids, with antioxidant effect, and the authors considered it as an advantage over traditional kombucha drink.

2.6. Methylsulfonylmethane

Methylsulfonylmethane (MSM) is an organic sulfur-containing compound that occurs naturally in a variety of plants and animals including humans. MSM is a normal oxidative metabolite product of dimethylsulfoxide (DMSO) and has been broadly used as a dietary supplement for the treatment of many conditions such as interstitial cystitis, snoring, allergic rhinitis, and osteoarthritis (Kim *et al.*, 2006). MSM is an aprotic solvent that can dissolve a wide range of solutes and is miscible with many solvents, and therefore can be added into many pharmaceutical preparations. Due to the structural similarity with DMSO, MSM is expected to act as a skin penetration enhancer for many drugs by similar mechanisms (Nishino *et al.*, 2008). In addition, some studies proposed that MSM forms bonds with hair follicle by delivering sulfur to the cortex layer of the hair, and promote the conversion of telogen to anagen phase or lengthen its anagen phase.

Chapter three

MATERIALS AND METHODS

3.Materials and Methods

3.1.Materials, chemicals and equipments:

Among the materials, chemicals and equipment used in the research are minoxidil (Dar Al Dawa, Jordan), sugar (Nader, Jordan), chamomile flowers (Kabatilo, Jordan), pure black tea (Ceylon tea, Siri Lanka), methyl sulfonyl methane (MSM) (Nutricost, China), hair removal cream Veet (Reckitt, France), glass jars (Mix glass, Jordan) and cotton (Cotton land, Jordan). Digital pH meter (SPMI Co.Ltd., China), incubator (LW Scientific, USA), autoclave (Alibaba, China), digital caliper (Rs PRO, China), electric shaver (Kemei, China), electric balance (CGOLDENWALL, China), camera EOS 80d (Canon, Japan) used with lense 18-135.

3.2.METHOD

3.2.1.Preparation of kombuch tea samples

Four different preparations of kombucha tea were prepared in accordance with previously described procedures (Dufresne and Farnworth, 2000) (Jayabalan *et al.*, 2014), with some modifications. All preparations, distinctive by different types of herbal tea and presence of MSM, were prepared under room temperature (20±1) and protected from direct light exposure. Details of experimental preparations are presented in the following sections:

3.2.1.1.Kombucha black tea Preparation and fermentation

Pure black tea (Ceylon tea, Siri Lanka) 10% was added to boiling water and allowed to infuse for 15 min; the infusion was then filtered through a sterile sieve. Sucrose 20% was added and dissolved in the above mixture, and the preparation was allowed to cool down to 25 °C.

The cooled mixture was poured into a glass jar sterilized at 121°C for 20 min, 3% (w/v) freshly grown tea fungus which was cultured with black tea for 14 days was introduced into the tea broth and 10% (v/v) of previously fermented liquid tea broth was added too. The Fermentation incubated in an incubator (LW Scientific, USA) at 25 °C covered with a clean cloth and tightly closed to prevent contamination and left for 15 days.

Figure 3. photograph for the fermentation of kombucha in black tea.

(By camera EOS 80d (Canon, Japan))

The fermentation was watched until the pH level drops to about 2.7 which were measured using digital pH meter (SPMI Co.Ltd., China), filtration was performed to the fermented mixture using a funnel with cotton to separate the filtrate from the symbiotic bacteria and yeast. The filtrate was then kept in another jar at subzero temperature to deactivate the fermenting bacteria and yeast in the liquor.

3.2.1.2.Kombucha chamomile tea preparation and fermentation:

Kombucha chamomile tea was prepared using the same procedure described in section 3.2.1.1 except that chamomile flowers were used instead of black tea.

Figure 4. photograph for the fermentation of kombucha in chamomile tea.

(A Pirzad *et al.*, 2006).

3.2.1.3.Preparation a mixture solution of kombucha black tea and MSM

A predetermined amount of MSM was added to an aliquot of kombucha black tea filtrate. The ratio of MSM: Kombucha black tea was 5% w/v.

3.2.1.4.Preparation a mixture solution of kombucha chamomile tea and MSM

A predetermined amount of MSM was added to an aliquot of kombucha chamomile tea filtrate. The ratio of MSM: Kombucha chamomile tea was 5% w/v.

3.3.Experimental

3.3.1.Experimental model for hair growth:

3.3.1.1.Selection and housing of experimental animals

A total of 48 adult male BALB/C mice were used in this part of the study. All mice were obtained from the animal house of Isra University and were of the same age 7 weeks, to make sure that all hair follicles were synchronized in the telogen phase (Rho *et al.*, 2005), the average weight around (26-33 gm). The mice were housed in plastic cages (30×30×20 cm) at room temperature (24±3) and 12 hrs light-12 hrs dark cycles. The mice were provided with a standard pellet diet and tap water ad libitum. All animal manipulations in the present study were conducted according to the guidelines of the Institutional Animal Care and Use Committee (IACUC).

3.3.1.2.Experimental design:

The mice were randomly distributed into eight groups (n=6/group) and were acclimatized for a period of 7 days. The animal groups were made distinctive by the topical treatments administrated to the mice as follows:

Group 1 (Negative control): Mice didn't receive any treatment.

Group 2 (Positive control): Mice were treated with a 2% minoxidil solution.

Group 3 (Black tea control): Mice were treated with black tea solution.

Group 4 (Chamomile tea control): Mice were treated with chamomile solution.

Group 5: Mice were treated with kombucha black tea.

Group 6: Mice were treated with kombucha chamomile tea

Group 7: Mice were treated with a mixture of kombucha black tea and MSM.

Group 8: Mice were treated with a mixture of kombucha chamomile tea and MSM.

Sprayed once daily with 3 puffs each.

3.3.1.3.Hair depilatory procedure

Following the acclimatization period, an area of the hair (4 cm2) from the dorsal part was shaved from each mouse using electrical razor and chemical depilation cream(Veet) and was then wiped with surgical spirit.

3.3.1.4.Assessment and evaluation of hair growth activity in mice

Evaluation of hair growth activity was performed according to procedures described previously (Park *et al.*, 2015). The mice were selected and grouped as describes in section 3.3.1.1. and 3.3.1.2., respectively. The back hair of the dorsal part was shaved according to the procedure described in section 3.3.1.3. The shaved area was then sprayed with 3 puffs of respective treatments, which was performed immediately after shaving and on a daily basis for 30 days. On days 1, 15, and 30 post-shaving, hair growth activity was determined at three test parameters:

Hair density over the shaved dorsal skin, Hair length and Hair initiation time.

Hair density was evaluated and analyzed statistically by scoring the area of hair growth by self-designed scales as described in Table 3. On the other hand, hair length was determined by plucking randomly, hair (n=7) from each mouse in the group with tweezers from the shaved area and measure their length via a digital caliper (Rs PRO, China) manually, and the results were recorded as mean ± SD of 42 hairs. Hair initiation time was evaluated and examined visually. Moreover, the shaved dorsal area was photographed daily using a high-resolution camera canon EOS 80d (Canon, Japan).

Table (3). Hair growth area score

Score	**Description**
1	No hair growth
2	less than 20% of hair growth
3	20-39% of hair growth
4	40-59% of hair regrowth
5	60-79% of hair regrowth
6	80-100% of hair

3.3.2.Statistical analysis

The results were presented as mean ± S.D. Data obtained was analyzed using the one-way Anova and Tukey's HSD test and differences with $P<0.05$ were considered statistically significant.

Chapter Four

RESULTS AND DISCUSSION

4.Results and Discussion

4.1.Result

Initially, the safety of the topical kombucha preparations used in this research was conducted before commencing the experiment by observing the mice visually for any sign of sensitivity reaction (such as redness, or swelling) for 72 hours by applying the mentioned preparations on the denuded area of the mice, which warranted them as safe to be applied topically.

Hair growth activity using balb/c mice, through which the mice were divided into 8 groups as mentioned in the experimental section, each group contains 6 mice were observed and photographed daily for 30 days by (camera EOS 80d Canon used with lense 18-135); a representative sample of one mice from day 1, 15 and 30 is presented in Fig 5-7 from the eight groups.

The first day showed that the dorsal part of the mice was hairless (Fig 5: hair density score 1). While on day 15, we noticed that (G5 and 7) promoted the growth of hair in the shaved area by almost 70%, while (G 2, 6 and 8) by around 40% of the total denuded area with obvious longer hair in groups (G5, 6, 7, and 8); however the vehicle control group (G3, and 4) especially the corresponding negative control group (G1) showed less noticeable, transient and irregular hair coats during this period. The whole denuded skin in the experimental mice had been fully covered by the hair when applied kombucha on day 30, however, group (G5, and 6) still had a small area with no hair and/or hair that is very short and not fully densely covered. This obviously stipulates that kombucha extract with and without MSM promoted the re-growth of hair, with the better results when combined with MSM.

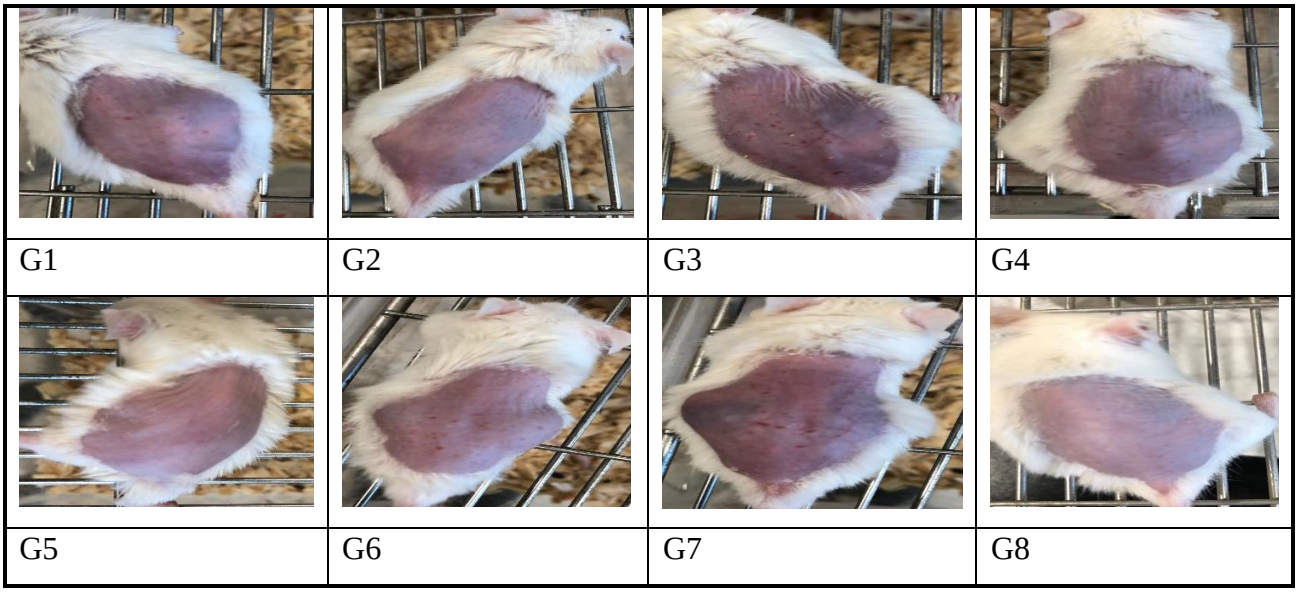

Figure 5. Photograph for hair growth for a representative mouse for each group after the 1st day of treatment: G7 (kombucha /black tea with MSM), G5 (kombucha/black tea), G3 (black tea), G8 (kombucha /chamomile tea with MSM), G6 (kombucha /chamomile tea), G4 (chamomile tea), G2 (minoxidil), G1 (distilled water).

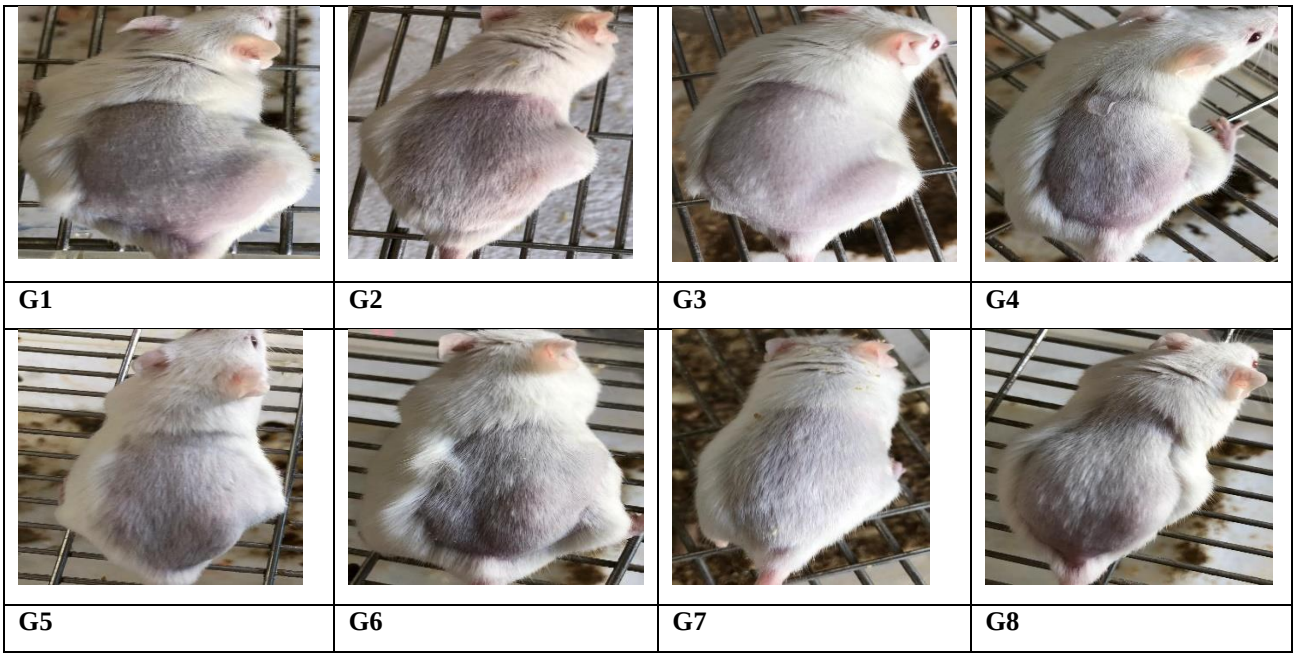

Figure 6. Photograph for hair growth for a representative mouse for each group after the 15th day of treatment: G7 (kombucha /black tea with MSM), G5 (kombucha/black tea), G3 (black tea), G8 (kombucha /chamomile tea with MSM), G6 (kombucha /chamomile tea), G4 (chamomile tea), G2 (minoxidil), G1 (distilled water).

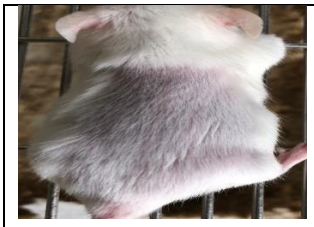	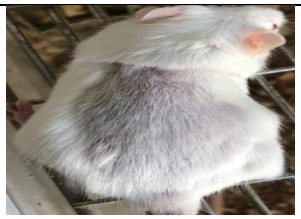	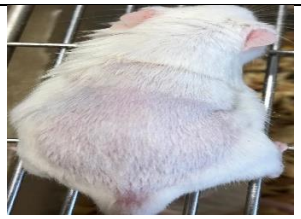	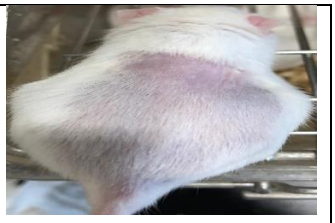
G1	G2	G3	G4
			
G5	G6	G7	G8

Figure 7. Photograph for hair growth for a representative mouse for each group after the 30th day of treatment: G7 (kombucha /black tea with MSM), G5 (kombucha/black tea), G3 (black tea), G8 (kombucha /chamomile tea with MSM), G6 (kombucha /chamomile tea), G4 (chamomile tea), G2 (minoxidil), G1 (distilled water).

4.1.1.Hair growth initiation time:

The minimum time for the growth of hair from the denuded dorsal area was observed and the time taken for a visible sign of hair was recorded in table 4. The results undoubtedly indicate that treatment with kombucha/black tea with MSM (G7) reduced the time for hair growth initiation by 7 days, however (G5, 6, and 8) reduced the time by 6 days, while the positive control group (G2) by 4 days which suggested the beneficial effect of kombucha in promoting hair growth and pushing the hair cycle to enter the anagen phase quickly. In comparison to the positive controlled group (G2), the time taken to start showing the visible sign of hair growth was after 10 days in compare to G7 which was after 7 days and for (G 5, 6 and 8) was after 8 days, the same was observed for the negative controlled vehicle groups (G 3, and 4) through which the time taken to show hair re-growth was after 12 days. Although some reduction in the time for initiation of hair re-growth was documented in the group (G3, and 4) but it was not as noticeable as with kombucha treated groups.

Table (4). Qualitative results for hair growth and the time taken to initiate hair growth.

GROUPS	Number of days for hair to initiate growth
G1	14
G2	10
G4	12
G3	12
G6	8
G8	8
G5	8
G7	7

*The time recorded after the first sign of hair initiation in the treated mice groups: G7 (kombucha /black tea with MSM), G5 (kombucha/black tea), G3 (black tea), G8 (kombucha /chamomile tea with MSM), G6 (kombucha /chamomile tea), G4 (chamomile tea), G2 (minoxidil), G1 (distilled water).

4.1.2.Effect of different kombucha tea extracts using black tea and chamomile with and without MSM on hair density:

The hair density analysis using the scoring system described in the method section (4.1.4) for day 15 and 30 are shown in table 5 and figures 8-9 respectively. The results presented in Table 5 indicated that there was a significant enhance in hair density approximately (70%, 50%, 38%, 20% P=0) after applying kombucha/ black tea with MSM, Kombucha/ black tea, kombucha/chamomile with MSM, kombucha/chamomile respectively and (P=0.0002) for minoxidil (G2) with respect to distilled water/ negative control group (G1) and 20% enhance in hair density with black tea (G3). However, no significant differences in hair density between chamomile tea (G4) and negative control (placebo) treated mice on day 15. However, the enhance in hair density was significant (P=0) for (G5,G7 and G8), (P=0.0056) for (G2) and (P=0.0001) for (G6) on day 30 except with the chamomile vehicle control group (G4) in comparison with the placebo group (G1). What is interesting that there was a significant enhancement (P=0) in hair density between mice treated group with kombucha/ black tea with MSM (G7) and Kombucha black tea (G5), (P=0.007) for (G8) and (P=0.0033) for (G4) in compare with positive control group/ minoxidil (G2) on day 15. But there are significant enhancement (P=0.0272 and P=0) for (G4 and G7) respectively in compare with positive control group/ minoxidil (G2) on day 30.

Table (5). The density of hair in the 15th and 30th day.

GROUPS	AVERAGE ± S.D for day 15	AVERAGE ± S.D for day 30
G1	1.45 ± 0.46	2.42 ± 0.66
G2	2.72 ± 0.43	3.92 ± 0.58
G4	1.67 ± 0.44	2.63 ± 0.63
G3	2.05 ± 0.39	3.30 ± 0.63
G6	3.03 ± 0.42	4.38 ± 0.53
G8	3.7 ± 0.49	4.70 ± 0.77
G5	4.15 ± 0.413	5.00 ± 0.71
G7	5.1 ± 0.4	6.05 ± 0.56

*Values are the mean ± S.D. based on the scoring system adopted from six mice in the treated mice group. For day 15 and 30: G7 (kombucha /black tea with MSM), G5 (kombucha/black tea), G3 (black tea), G8 (kombucha /chamomile tea with MSM), G6 (kombucha /chamomile tea), G4 (chamomile tea), G2 (minoxidil), G1 (distilled water), compared with G1.

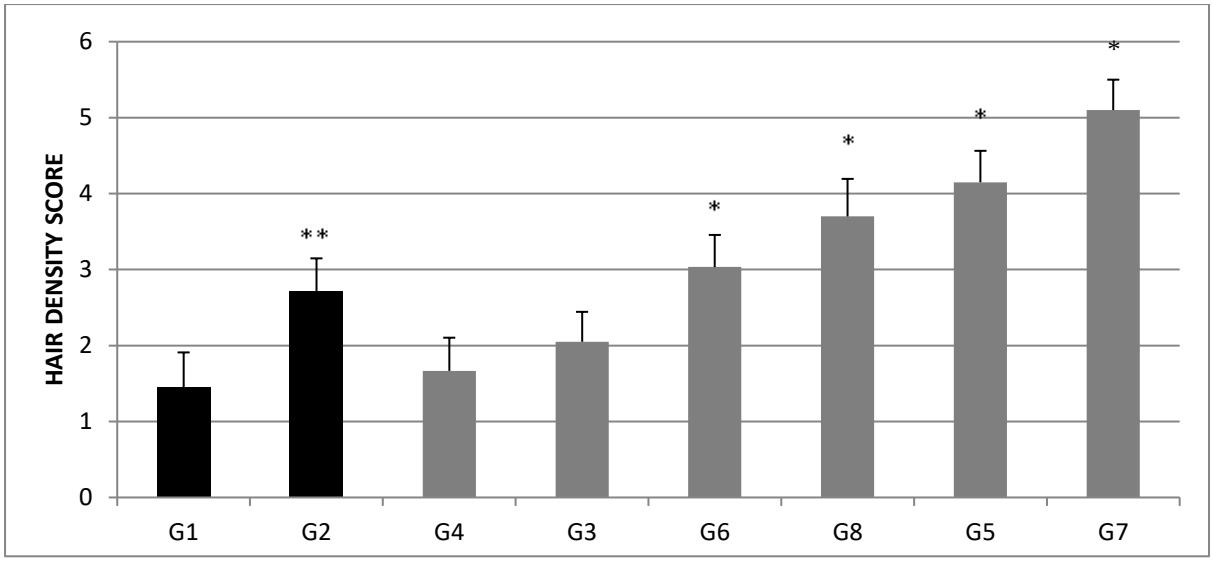

Figure 8. Hair density activity based on the scoring system adopted from six mice in the treated mice group on day 15**.** Values in are means ± S.D. based on the scoring system for : G7 (kombucha /black tea with MSM), G5 (kombucha/black tea), G3 (black tea), G8 (kombucha /chamomile tea with MSM), G6 (kombucha /chamomile tea), G4 (chamomile tea), G2 (minoxidil), G1 (distilled water), compared with G1.
(*,**,***) Indicate significant differences than control group where *P<0.05, **P<0.001, ***P<0.0001.

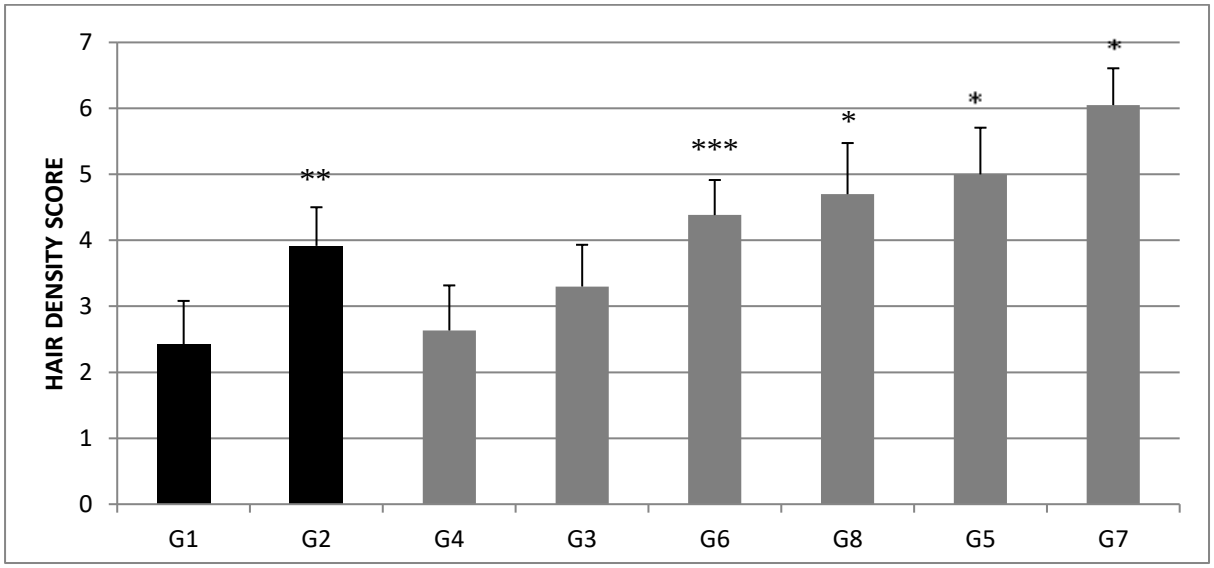

Figure 9. Hair density activity based on the scoring system adopted from six mice in the treated mice group on day 30**.** Values in are means ± S.D. based on the scoring system for : G7 (kombucha /black tea with MSM), G5 (kombucha/black tea), G3 (black tea), G8 (kombucha /chamomile tea with MSM), G6 (kombucha /chamomile tea), G4 (chamomile tea), G2 (minoxidil), G1 (distilled water), compared with G1.
(*,**,***) Indicate significant differences than control group where *P<0.05, **P<0.001, ***P<0.0001.

4.1.3.Effect of different kombucha tea extracts using black tea and chamomile with and without MSM on hair length:

The hair length analysis using caliper described in the method section 4.1.4. for black tea and chamomile for days, 15 and 30 are shown in table 6 and figure 10-11 respectively. The results presented in Table 6 indicated that there was a significant enhancement (*P*=0) for (G5,G7 and G8), (P=0.0336) for (G2) and (P=0.0003) for (G6) in the length of hair except with (G4 and G3) in comparison with the negative control group on day 15. And there was a significant enhancement (P=0) for (G2,G5,G6,G7 and G8) in the length of hair except with (G4 and G3) in comparison with the negative control group on day 30. The length of hair was tripled for G7 (9.3 ± 0.87, 14.3 ± 0.77) compared with the length of hair in the negative control group (G1) (3.7 ± 0.94, 5.9 ± 0.98) on the 15th and 30th day respectively, which indicates that both kombucha and MSM had an extraordinary effect on the length of the hair, which means enhancing the growth phase duration. The results explored that the vehicle control group black tea showed a significant enhance in the length of the hair fiber, while not significant for chamomile vehicle control group (G4) on comparison with the negative control group (G1), which indicates that black tea had an effect on hair growth and length but not important with respect to chamomile tea. Worth to mention that the length of hair for the treated mice with kombucha black tea (G5) was significantly better (*P*=0.0012) than the positive control (minoxidil) treated group on both days 15 and 30. The length of hair for the treated mice with kombucha black tea and MSM (G7) was significantly better (*P*=0) than the positive control (minoxidil) treated group on both days 15 and 30. The length of hair for the treated mice with (G3 and G4) was significantly better (*P*=0.0202 and P=0) respectively than the positive control (minoxidil) treated group on day 30. which is found to be inconsistent with the results obtained for hair density with kombucha in compare to the negative control group and positive control (minoxidil) group.

Table 6: Hair length in day 15 and 30 .

GROUPS	AVERAGE ± S.D for day 15	AVERAGE ± S.D for day 30
G1	3.66 ± 0.94	5.90 ± 0.98
G2	5.77 ± 1.08	9.73 ± 1.04
G4	4.33 ± 0.75	6.00 ± 0.96
G3	4.92 ± 1.00	7.42 ± 1.48
G6	6.80 ± 1.13	10.80 ± 1.13
G8	7.73 ± 1.01	11.73 ± 1.04
G5	8.60 ± 1.04	12.70 ± 0.99
G7	9. 33 ± 0.87	14.25 ± 0.77

*Values in (mm) are means ± S.D. from six mice in each group. G7 (kombucha /black tea with MSM), G5 (kombucha/black tea), G3 (black tea), G8 (kombucha /chamomile tea with MSM), G6 (kombucha /chamomile tea), G4 (chamomile tea), G2 (minoxidil), G1 (distilled water), Compared with G1.

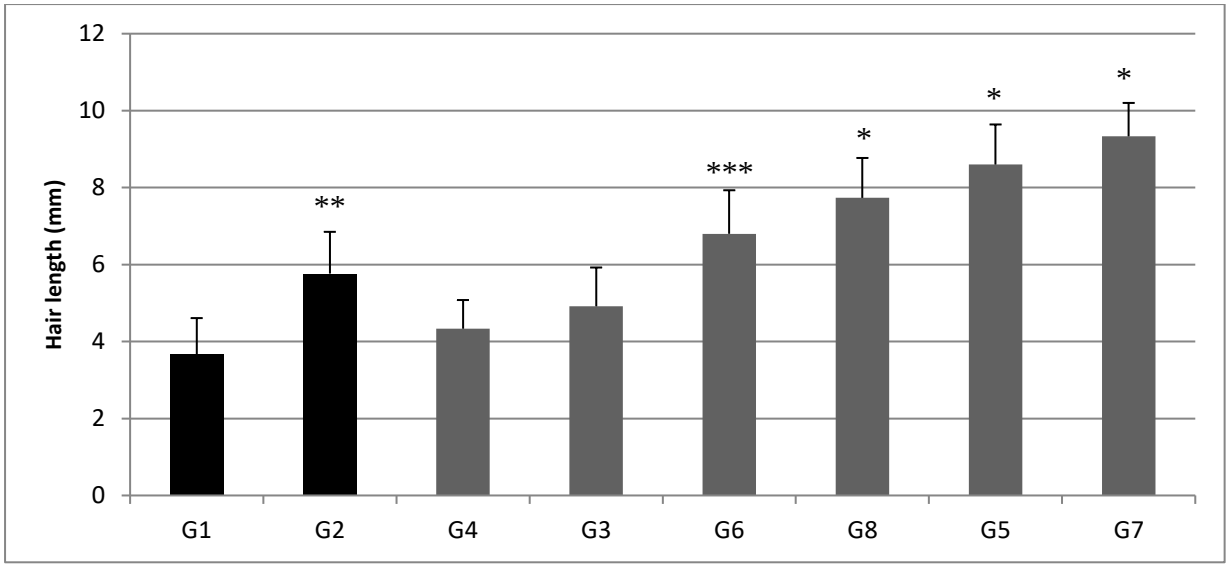

Figure 10. Values in (mm) for the length of hair are means ± S.D. from six mice in each group, on day 15: G7 (kombucha /black tea with MSM), G5 (kombucha/black tea), G3 (black tea), G8 (kombucha /chamomile tea with MSM), G6 (kombucha /chamomile tea), G4 (chamomile tea), G2 (minoxidil), G1 (distilled water), Compared with G1.

(*,**,***) Indicate significant differences than control group where *P<0.05, **P<0.001, ***P<0.0001.

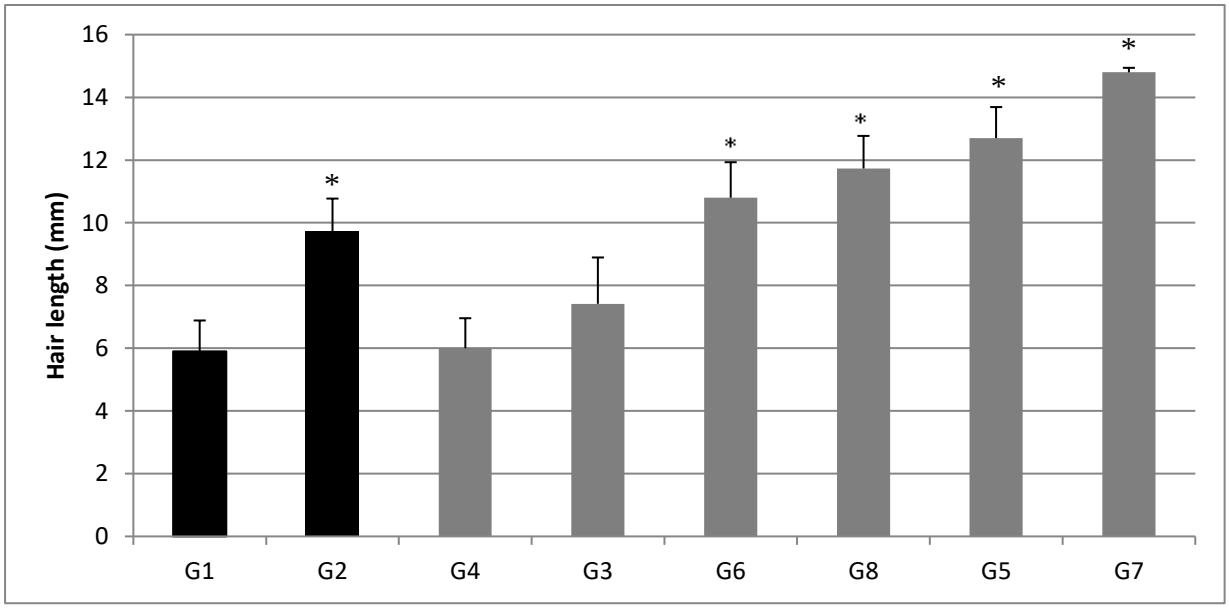

Figure 11. Values in (mm) for the length of hair are means ± S.D. from six mice in each group, on day 30: G7 (kombucha /black tea with MSM), G5 (kombucha/black tea), G3 (black tea), G8 (kombucha /chamomile tea with MSM), G6 (kombucha /chamomile tea), G4 (chamomile tea), G2 (minoxidil), G1 (distilled water), Compared with G1.

(*,**,***) Indicate significant differences than control group where *P<0.05, **P<0.001, ***P<0.0001.

4.2.Discussion

The results of this study demonstrated the potential effect of kombucha extract with MSM in denuded mice as a hair re-growth natural agent (Figures 6 and 7). These observations could be explained by understanding the mechanism through which alopecia occurs, which is summarized by the following purported mechanism:

Disruption or perturbation of the hair cycle; which is the primary mechanism for alopecia (Wolff *et al.*, 2017). Therefore, delayed onset or lessen the duration of the active phase and prolonged the resting phase period are prevalent mechanisms recognized in hair loss.

Irregularities in molecular communication during the morphological phases (Breitkopf *et al.*, 2013). Lastly, Loss of epithelial stem cells (Breitkopf *et al.*, 2013).

There are several means through which a drug affects the regeneration of hair, either by enhancing the hair diameter; change the cycle of the hair by prolonging its anagen phase; enhance the growth and density of hair, or through a combination of these effects. Kombucha extract in the present study selected based on its use in traditional medicines for much heath disorder including hair care but never been tested topically or proved orally. Our research with kombucha which is a symbiotic acetobacter bacterium (Acetobacter) and yeast (*ZygoSaccharomyces bailli*) fermented in sugared tea (black tea and chamomile), generated different organic acids, vitamins (B, and C), ethanol, minerals, and amino acids. All these bioactive compounds have been shown in the literature to have numerous beneficial effects for the enhancement and regeneration of hair growth and this explains their effect as a powerful natural agent to combat hair loss. In this study, we explored the promoting effect of hair growth for topical application of kombucha fermented with black and chamomile tea with and without MSM, on denuded mice skin (Shanmugam *et al.*, 2009). This naturally fermented kombucha produces plentiful bioactive components, and their advantageous and beneficial application could speed up hair growth and regeneration. This fermented kombucha contained high levels of water-soluble vitamins, particularly niacin, pyridoxine and ascorbic acid, that function as an antioxidant which reduces the oxidative stress and nourishes the dermal papilla cell thus revealed the ability of kombucha extract to enhance the growth of hair resulted in promoting hair growth in our mice model. It was distinguished that the treatment with kombucha extract resulted in expanded the length of the active phase (anagen) of the hair cycle, compared to the control group. In addition, the capability of kombucha extract to

promote the growth of hair in denuded mice, when compared to the control group as measured by different parameters, including hair initiation time, hair density, and hair length analysis. Obviously the explored treatment with kombucha extract contains bioactive components such as thiamine (Vit B1) that had a vasodilating effect which enhances the blood flow to the scalp, and 5-α-reductase inhibitor (Semalty *et al.*, 2011); pyridoxine (Vit B6) act as nutrients to nourish the roots of the hair follicle and is consumed by the hair follicle to improve their usage of amino acid cysteine the building block for hair fibers, in addition, helps in giving rise to a shiny stronger and thicker hair fiber (Rogers and Avram, 2008); riboflavin (Vit B2) found to stimulate the Vit B6 through the conversion of tryptophan to niacin and plays a role in the production of glutathione thus reduces the oxidative stress associated with free radical on hair follicles (Yan *et al.*, 2016). Cobalamin (Vit B12) important vitamin for hair quality by helping in the production of red blood cells rich with oxygen, which feed hair follicles; ascorbic acid (Vit C) that maintains capillaries conveying blood flow to the hair follicle, therefore, enhancing blood flow to scalp; zinc (Zn) which acts as an immunomodulator to the follicle, consider an important cofactor for many enzymes involved in an essential function in the activity of the hair follicle (Almohanna *et al.*, 2019), has a role in RNA and DNA production which is a prerequisite for a systemized division of hair follicle cells which enhances the anagen phase of the hair cycle (Park *et al.*, 2009), in addition, it is 5-α-reductase inhibitor thus impedes the conversion of testosterone to DHT (Hosking, Juhasz and Atanaskova Mesinkovska, 2019), finally, the kombucha treatment contains many essential amino acids that enhances the hair texture quality providing rigidity and strength between the strands of keratin.

Another important component is iron (Fe) which explicated by many authors that its deficiency might alter the normal development of the hair cycle and leads to androgenic alopecia and telogen effluvium (Almohanna *et al.*, 2019).

Other components of kombucha extract in this study are lactic acid and acetic acid (both are alpha hydroxyl acid {AHA}) that suggested acting as exfoliants which normalizes the keratinocyte accumulation or keratinization process on the hair follicle that enhances the hair stand emerging through the scalp because imperfect keratinization in the hair shaft causes the hair to break off at the skin level (Rigdon and Packchanian, 1974); and irritants, which hypothesized to encourage angiogenesis and enhances blood flow to the scalp, therefore, promoting the exchange of nutrition in the follicle resulting in superior hair growth with enhance in density and aspect (Woo *et al.*, 2019). In addition to AHA found

in the kombucha extract, gluconic Acid which is a polyhydroxy acid, the second generation of AHA was found in this fruitful extract which plays a role in hair re-growth by its irritant and angiogenesis effect (Rigdon and Packchanian, 1974).

In our research it was explored that kombucha fermented in black tea gave a better result in hair promoting effect than with chamomile fermented kombucha, even black tea as a vehicle showed some beneficial effect, but was not as pronounced as with kombucha, compared with the negative control group, this can be proposed by the presence of a beneficial component in black tea that promoted hair growth such as caffeine and flavonoids. Fischer et al. reported that caffeine found to inhibit phosphodiesterase, that encourages cell proliferation in the hair matrix by increasing the level of cAMP in the cell and thus stimulate its metabolism, this would impede the negative effect of DHT in the hair follicle (Bansal, Manchanda and Pandey, 2012) (Mahajan and Pandey, 2018), therefore lengthen the growth phase time span and promotes the elongation of hair fiber (Fischer, Hipler and Elsner, 2007). Additionally, Bansal et al. reported that caffeine down-regulates the expression of an inhibitor of hair growth which is testosterone- induced transforming growth factor (TGF) B1, which counteract the effect of DHT in the hair follicle (Fischer *et al.*, 2014). However, flavanoids of black tea such as EGCG investigated by Hou et al. to have a high affinity for estrogen alpha receptor and since its hair growth-promoting activity (Hou *et al.*, 2013).

Lastly, MSM contributed to promoting hair growth, which is an organic sulfur-containing compound that occurs naturally in a variety of plants and animals including humans. MSM is a normal oxidative metabolite product of dimethylsulfoxide (DMSO) and has been broadly used as a dietary supplement for the treatment of many conditions such as interstitial cystitis, snoring, allergic rhinitis, and osteoarthritis (Kim *et al.*, 2006). MSM is an aprotic solvent that can dissolve a wide range of solutes and is miscible with many solvents, and therefore can be added into many pharmaceutical preparations. Due to the structural similarity with DMSO, MSM is expected to act as a skin penetration enhancer for many drugs by similar mechanisms and play a major role in drug delivery technology (Nishino *et al.*, 2008). In addition, some studies proposed that MSM forms bonds with hair follicle by delivering sulfur to the cortex layer of the hair, and promote the conversion of telogen to anagen phase or lengthen its anagen phase (Shanmugam *et al.*, 2009).

Chapter Five

CONCLUSIONS AND RECOMMENDATIONS

5. Conclusions and recommendations

5.1.Conclusions

From this research of *in vivo* hair growth activity examination, it can be strongly proposed that kombucha fermentation in black tea has bioactive components to promote, restore the growth of hair through elongating the anagen phase of the mice model and improving blood flow to the scalp.

5.2.Recommendations

This study recommends the possible potential of kombucha extracts to be used as a natural alternative therapy for hair loss. Generally, this study encourages including kombucha into dietary patterns to prevent or treat hair loss. Overall, the use of foods with different bioactive compounds not only to prevent and manage hair loss but also for other health matters. As a whole, this natural alternative therapy as kombucha extract is not only cost-effective but definitely has fewer side effects compared to the more expensive synthetic drugs.

Chapter Six

References

Adhirajan, N. *et al.* (2003) 'In vivo and in vitro evaluation of hair growth potential of Hibiscus rosa-sinensis Linn.', *Journal of Ethnopharmacology*, 88(2–3), pp. 235–239. doi: 10.1016/S0378-8741(03)00231-9.

Adhirajan, N., DIXIT, V. K. and CHANDRAKASAN, G. (2001) 'Development and evaluation of herbal formulations for hair growth', *Indian drugs*. Indian Drug Manufacturers' Association, 38(11), pp. 559–563.

Aldhalimi, M. A., Hadi, N. R. and Ghafil, F. A. (2014) 'Promotive effect of topical ketoconazole, minoxidil, and minoxidil with tretinoin on hair growth in male mice', *International Scholarly Research Notices*. Hindawi, 2014.

Almohanna, H. M. *et al.* (2019) 'The Role of Vitamins and Minerals in Hair Loss: A Review', *Dermatology and Therapy*. Springer Healthcare, 9(1), pp. 51–70. doi: 10.1007/s13555-018-0278-6.

Aloulou, A. *et al.* (2012) 'Hypoglycemic and antilipidemic properties of kombucha tea in alloxan-induced diabetic rats', *BMC Complementary and Alternative Medicine*, 12. doi: 10.1186/1472-6882-12-63.

Amarasinghe, H., Weerakkody, N. S. and Waisundara, V. Y. (2018) 'Evaluation of physicochemical properties and antioxidant activities of kombucha "Tea Fungus" during extended periods of fermentation', *Food Science and Nutrition*, 6(3), pp. 659–665. doi: 10.1002/fsn3.605.

Bansal, M., Manchanda, K. and Pandey, S. (2012) 'Role of caffeine in the management of androgenetic alopecia', *International journal of trichology*. Medknow Publications & Media Pvt. Ltd., 4(3), p. 185.

Barrera, A. (2005) 'Hair restoration', *Clinics in Plastic Surgery*, 32(2), pp. 163–170. doi: 10.1016/j.cps.2004.11.007.

Blume-Peytavi, U. *et al.* (2012) 'A randomized double-blind placebo-controlled pilot study to

assess the efficacy of a 24-week topical treatment by latanoprost 0.1% on hair growth and pigmentation in healthy volunteers with androgenetic alopecia', *Journal of the American Academy of Dermatology*. Elsevier, 66(5), pp. 794–800.

Breitkopf, T. *et al.* (2013) 'The Basic Science of Hair Biology. What Are the Causal Mechanisms for the Disordered Hair Follicle?', *Dermatologic Clinics*, 31(1), pp. 1–19. doi: 10.1016/j.det.2012.08.006.

Buhl, A. E. *et al.* (1993) 'Potassium channel conductance as a control mechanism in hair follicles', *Journal of investigative dermatology*. Elsevier, 101(1), pp. S148–S152.

Carmina, E. and Lobo, R. A. (2003) 'Treatment of hyperandrogenic alopecia in women', *Fertility and sterility*. Elsevier, 79(1), pp. 91–95.

Cohen, J. L. (2010) 'Enhancing the growth of natural eyelashes: the mechanism of bimatoprost-induced eyelash growth', *Dermatologic surgery*. Wiley Online Library, 36(9), pp. 1361–1371.

Coneac, A., Muresan, A. and Orasan, M. S. (2014) 'Antiandrogenic therapy with ciproterone acetate in female patients who suffer from both androgenetic alopecia and acne vulgaris', *Clujul Medical*. Universty of Medicine and Pharmacy of Cluj-Napoca, Romania, 87(4), p. 226.

Deghrigue, M. *et al.* (2013) 'Antiproliferative and antimicrobial activities of kombucha tea', *African Journal of Microbiology Research*, 7(27), pp. 3466–3470. doi: 10.5897/AJMR12.1230.

Diani, A. R. *et al.* (1992) 'Hair growth effects of oral administration of finasteride, a steroid 5 alpha-reductase inhibitor, alone and in combination with topical minoxidil in the balding stumptail macaque', *The Journal of Clinical Endocrinology & Metabolism*. Oxford University Press, 74(2), pp. 345–350.

Dufresne, C. and Farnworth, E. (2000) 'Tea, Kombucha, and health: a review', *Food research international*. Elsevier, 33(6), pp. 409–421.

Esfandiari, A. and Kelly, A. P. (2005) 'The effects of tea polyphenolic compounds on hair loss among rodents.', *Journal of the National Medical Association*. National Medical Association, 97(8), p. 1165.

Eun, H. C. *et al.* (2010) 'Efficacy, safety, and tolerability of dutasteride 0.5 mg once daily in male patients with male pattern hair loss: a randomized, double-blind, placebo-controlled, phase III study', *Journal of the American Academy of Dermatology*. Elsevier, 63(2), pp. 252–258.

Faghihi, G., Andalib, F. and Asilian, A. (2012) 'The efficacy of latanoprost in the treatment of alopecia areata of eyelashes and eyebrows.', *Journal of Isfahan Medical School*, 29(172).

Fiedler, V. C. (1992) 'Alopecia areata: a review of therapy, efficacy, safety, and mechanism', *Archives of dermatology*. American Medical Association, 128(11), pp. 1519–1529.

Fischer, T. W. *et al.* (2014) 'Differential effects of caffeine on hair shaft elongation, matrix and outer root sheath keratinocyte proliferation, and transforming growth factor-β2/insulin-like growth factor-1-mediated regulation of the hair cycle in male and female human hair follicle', *British Journal of Dermatology*, 171(5), pp. 1031–1043. doi: 10.1111/bjd.13114.

Fischer, T. W., Hipler, U. C. and Elsner, P. (2007) 'Effect of caffeine and testosterone on the proliferation of human hair follicles in vitro', *International Journal of Dermatology*, 46(1), pp. 27–35. doi: 10.1111/j.1365-4632.2007.03119.x.

Galati, A. *et al.* (2014) 'The Cereal Market and the Role of Fermentation in Cereal-Based Food Production in Africa', *Food Reviews International*, 30(4), pp. 317–337. doi: 10.1080/87559129.2014.929143.

Goldberg, M. R. (1988) 'Clinical pharmacology of pinacidil, a prototype for drugs that affect potassium channels', *Journal of cardiovascular pharmacology*. LWW, 12, pp. S41–S47.

Harmon, C. S., Lutz, D. and Ducote, J. (1993) 'Potassium channel openers stimulate DNA synthesis in mouse epidermal keratinocyte and whole hair follicle cultures', *Skin Pharmacology and Physiology*. Karger Publishers, 6(3), pp. 170–178.

Hosking, A. M., Juhasz, M. and Atanaskova Mesinkovska, N. (2019) 'Complementary and Alternative Treatments for Alopecia: A Comprehensive Review', *Skin Appendage Disorders*, pp. 72–89. doi: 10.1159/000492035.

Hou, I. C. *et al.* (2013) 'A hair growth-promoting effect of Chinese black tea extract in mice', *Bioscience, Biotechnology and Biochemistry*, 77(7), pp. 1606–1607. doi: 10.1271/bbb.130298.

Jarrell, J., Cal, T. and Bennett, J. W. (2000) 'The Kombucha consortia of yeasts and bacteria', *Mycologist*. Elsevier, 14(4), pp. 166–170.

Jayabalan, R. *et al.* (2014) 'A review on kombucha tea—microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus', *Comprehensive Reviews in Food Science and Food Safety*. Wiley Online Library, 13(4), pp. 538–550.

Kaushik, R., Gupta, D. and Yadav, R. (2011) 'Alopecia: herbal remedies', *International Journal of Pharmaceutical Sciences and Research*. International Journal of Pharmaceutical Sciences and Research, 2(7), p. 1631.

Kim, L. S. *et al.* (2006) 'Efficacy of methylsulfonylmethane (MSM) in osteoarthritis pain of the knee: a pilot clinical trial', *Osteoarthritis and Cartilage*. Elsevier, 14(3), pp. 286–294.

Kwon, O. S. *et al.* (2007) 'Promotive effect of minoxidil combined with all-trans retinoic acid (tretinoin) on human hair growth in vitro', *Journal of Korean medical science*, 22(2), pp. 283–289.

Leal, J. M. *et al.* (2018) 'A review on health benefits of kombucha nutritional compounds and metabolites', *CyTA-Journal of Food*. Taylor and Francis Ltd, 16(1), pp. 390–399.

Levy, L. L. and Emer, J. J. (2013) 'Female pattern alopecia: current perspectives', *International journal of women's health*. Dove Press, 5, p. 541.

Mahajan, S. and Pandey, S. S. (2018) 'Reinterpreting minimum inhibitory concentration (MIC)

data of itraconazole versus terbinafine for dermatophytosis-time to look beyond the MIC data? Reply', *INDIAN JOURNAL OF DERMATOLOGY VENEREOLOGY & LEPROLOGY*. MEDKNOW PUBLICATIONS & MEDIA PVT LTD B-9, KANARA BUSINESS CENTRE, OFF LINK …, 84(1), pp. 63–64.

Messenger, A. G. and Rundegren, J. (2004) 'Minoxidil: mechanisms of action on hair growth', *British journal of dermatology*. Wiley Online Library, 150(2), pp. 186–194.

Nakaoji, K. *et al.* (1997) 'Bisbenzylisoquinoline alkaloids from Stephania cepharantha and their effects on proliferation of cultured cells from the murine hair apparatus', *Planta medica*. © Georg Thieme Verlag Stuttgart· New York, 63(05), pp. 425–428.

Nishino, Y. *et al.* (2008) 'Glycogen synthase kinase-3 inactivation is not required for ischemic preconditioning or postconditioning in the mouse', *Circulation research*. Am Heart Assoc, 103(3), pp. 307–314.

Olsen, E. A. *et al.* (2006) 'The importance of dual 5α-reductase inhibition in the treatment of male pattern hair loss: results of a randomized placebo-controlled study of dutasteride versus finasteride', *Journal of the American Academy of Dermatology*. Elsevier, 55(6), pp. 1014–1023.

Osawa, Y. *et al.* (2004) 'Promotion of hair-growth with Laminaria angustata extracts', *International Journal of Cosmetic Science*. Wiley Online Library, 26(4), p. 215.

Park, G. H. *et al.* (2015) 'Red ginseng extract promotes the hair growth in cultured human hair follicles', *Journal of Medicinal Food*, 18(3), pp. 354–362. doi: 10.1089/jmf.2013.3031.

Park, H. *et al.* (2009) 'The therapeutic effect and the changed serum zinc level after zinc supplementation in alopecia areata patients who had a low serum zinc level', *Annals of Dermatology*, 21(2), pp. 142–146. doi: 10.5021/ad.2009.21.2.142.

Pirzad, A. *et al.* (2006) 'Essential oil content and composition of German chamomile (Matricaria chamomilla L.) at different irrigation regimes', *Journal of Agronomy*, 5(3), pp. 451–455. doi: 10.3923/ja.2006.451.455.

Pirzad, A *et al.* (2006) 'Essential oil content and composition of German chamomile (Matricaria chamomilla L.) at different irrigation regimes', *Journal of Agronomy*. Asian Network for Scientific Information, 5(3), pp. 451–455.

Rho, S.-S. *et al.* (2005) 'The hair growth promoting effect of Asiasari radix extract and its molecular regulation', *Journal of Dermatological Science*. Elsevier, 38(2), pp. 89–97.

Rigdon, R. H. and Packchanian, A. N. A. (1974) 'Histological study of the athymic "nude" mice. Te. z. Rep', *Biol. Med*, 32, pp. 711–732.

Robertson, D. W. and Steinberg, M. I. (1990) 'Potassium channel modulators: scientific applications and therapeutic promise', *Journal of medicinal chemistry*. ACS Publications, 33(6), pp. 1529–1541.

Roenigk Jr, H. H. (1988) 'New topical agents for hair growth', *Clinics in Dermatology*. Elsevier, 6(4), pp. 119–121.

Rogers, N. E. and Avram, M. R. (2008) 'Medical treatments for male and female pattern hair loss', *Journal of the American Academy of Dermatology*, 59(4), pp. 547–566. doi: 10.1016/j.jaad.2008.07.001.

Roy, Ram Kumar, Thakur, M. and Dixit, V. K. (2007) 'Development and evaluation of polyherbal formulation for hair growth–promoting activity', *Journal of cosmetic dermatology*. Wiley Online Library, 6(2), pp. 108–112.

Roy, R K, Thakur, M. and Dixit, V. K. (2007) 'Effect of citrullus colocynthis. On hair growth in albino rats', *Pharmaceutical biology*. Taylor & Francis, 45(10), pp. 739–744.

Rushton, D. H. *et al.* (2002) 'Causes of hair loss and the developments in hair rejuvenation', *International journal of cosmetic science*. Wiley Online Library, 24(1), pp. 17–23.

Sakaguchi, I. *et al.* (2004) 'The water-soluble extract of Illicium anisatum stimulates mouse

vibrissae follicles in organ culture', *Experimental dermatology*. Wiley Online Library, 13(8), pp. 499–504.

Sastre, J. and Sabater, L. (2005) 'Fisiología de la secreción pancreática', 28(Supl 2), pp. 3–9.

Scalbert, A., Johnson, I. T. and Saltmarsh, M. (2005) 'Polyphenols: antioxidants and beyond', *The American journal of clinical nutrition*. Oxford University Press, 81(1), pp. 215S-217S.

Semalty, M. *et al.* (2011) 'Hair growth and rejuvenation: An overview', *Journal of Dermatological Treatment*, 22(3), pp. 123–132. doi: 10.3109/09546630903578574.

Setorkil, M. and Ahangar Darabi, M. (2013) 'Protective effects of kombucha tea and silimarin against thioacetamide induced hepatic injuries in wistar rats', *World Applied Sciences Journal*, 27(4), pp. 524–532.

Shanmugam, S. *et al.* (2009) 'The effect of methylsulfonylmethane on hair growth promotion of magnesium ascorbyl phosphate for the treatment of alopecia', *Biomolecules and Therapeutics*, 17(3), pp. 241–248. doi: 10.4062/biomolther.2009.17.3.241.

Singh, O. *et al.* (2011) 'Chamomile (Matricaria chamomilla L.): an overview', *Pharmacognosy reviews*. Wolters Kluwer--Medknow Publications, 5(9), p. 82.

Sun, T. Y., Li, J. S. and Chen, C. (2015) 'Effects of blending wheatgrass juice on enhancing phenolic compounds and antioxidant activities of traditional kombucha beverage', *Journal of Food and Drug Analysis*. Elsevier Ltd, 23(4), pp. 709–718. doi: 10.1016/j.jfda.2015.01.009.

Tosti, A. and Duque-Estrada, B. (2009) 'Treatment strategies for alopecia', *Expert opinion on pharmacotherapy*. Taylor & Francis, 10(6), pp. 1017–1026.

Uno, H. *et al.* (2002) 'Effect of latanoprost on hair growth in the bald scalp of the stump-tailed macacque: a pilot study.', *Acta dermato-venereologica*, 82(1).

Vázquez-Cabral, B. D. *et al.* (2017) 'Oak kombucha protects against oxidative stress and inflammatory processes', *Chemico-Biological Interactions*. Elsevier, 272, pp. 1–9.

Vexiau, P. *et al.* (2002) 'Effects of minoxidil 2% vs. cyproterone acetate treatment on female androgenetic alopecia: a controlled, 12-month randomized trial', *British Journal of Dermatology*. Wiley Online Library, 146(6), pp. 992–999.

Watawana, M. I. *et al.* (2015) 'Health, wellness, and safety aspects of the consumption of kombucha', *Journal of Chemistry*. Hindawi, 2015.

Wolff, K. *et al.* (2017) *Fitzpatrick's color atlas and synopsis of clinical dermatology*. McGraw-Hill,.

Woo, Y. M. *et al.* (2019) 'The effect of Lactobacillus plantarum hydrolysates promoting VEGF production on vascular growth and hair growth of C57BL/6 mice', *Journal of Analytical Science and Technology*. Journal of Analytical Science and Technology, 10(1). doi: 10.1186/s40543-019-0178-0.

Wood, A. J. and Price, V. H. (1999) 'Treatment of hair loss', *N Engl J Med*, 341(13), pp. 964–973.

Yan, Y. *et al.* (2016) 'Is nutrient intake associated with physical activity levels in healthy young adults?', *Public Health Nutrition*, 19(11), pp. 1983–1989. doi: 10.1017/S1368980015003717.

Yazdabadi, A. and Sinclair, R. (2011) 'Treatment of female pattern hair loss with the androgen receptor antagonist flutamide', *Australasian journal of dermatology*. Wiley Online Library, 52(2), pp. 132–134.

Zaccheo, T., Giudici, D. and Di Salle, E. (1998) 'Effect of the dual 5α-reductase inhibitor Pnu 157706 on the growth of dunning R3327 prostatic carcinoma in the rat', *The Journal of steroid biochemistry and molecular biology*. Elsevier, 64(3–4), pp. 193–198.

تقييم إمكانات نمو الشعر من مستخلصات الكمبوتشا موضعياً في الفئران

اعداد
محمد اياد صالح

المشرف

د.منال النجداوي

المشرف المساعد
د.قيس جرار

ملخص

يُعتقد أن استهلاك الأطعمة والمشروبات الوظيفية مثل شاي الكمبوتشا يعطي العديد من الآثار المفيدة لتعزيز المناعة والوقاية من السرطان. أجريت هذه الدراسة للتحقيق في إمكانات نمو الشعر من شاي الكمبوتشا الأسود وشاي البابونج مع وبدون ميثيل سلفونيل ميثان (MSM) في الجسم الحي باستخدام فئران Balb c /. تم تقسيم الفئران إلى ثماني مجموعات. مينوكسيديل 2٪ كمجموعة تحكم إيجابية ، والماء المقطر كمجموعة تحكم سلبية ، والشاي الأسود وشاي البابونج كمجموعة تحكم ، ومجموعات الاختبار التي تشمل كومبوتشا المخمرة في الشاي الأسود مع وبدون MSM ، الكمبوتشا المخمرة في شاي البابونج مع و بدون MSM على التوالي. تم تطبيق المستحضرات موضعياً على الفئران وتمت ملاحظتها لمدة 30 يومًا. لوحظ وقت بدء نمو الشعر لجميع المجموعات ، وأظهرت النتائج أن العلاج باستخدام الكومبوتشا / الشاي الأسود مع مكمل MSM الغذائي قلل من وقت بدء نمو الشعر بمقدار 7 أيام. تم تقييم كثافة الشعر وأوضحت النتائج أن هناك تحسناً معنوياً في كثافة الشعر بنسبة 70٪ تقريباً ($P < 0.0001$) بعد رش الكمبوتشا / الشاي الأسود مع MSM. تم قياس طول الشعر على فترتين في اليوم الخامس عشر والثلاثين. أظهرت النتائج استطالة معنوية للشعر بقيمة ($p < 0.0001$) لمجموعة الفئران التي عولجت بشاي كومبوتشا الأسود مع MSM.

من الدراسة الحالية يمكننا أن نستنتج أن الكمبوتشا المخمرة في الشاي الأسود مع MSM يمكن اعتبارها علاجًا بديلاً طبيعيًا وفعالًا لتساقط الشعر ، بالإضافة إلى علاج فعال من حيث التكلفة مع آثار جانبية أقل مقارنة بالأدوية الاصطناعية.