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RESEARCH ARTICLE

Evaluation of hair growth properties of Topical Kombucha tea extracts

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Abstract
Consuming functional foods and drinks such as kombucha tea is believed to give many beneficial effects for promoting immunity and preventing cancer. The fermented tea with sugar and symbiotic culture of yeast and bacteria is also useful in the topical application for hair re-growth. This study aims to investigate hair re-growth properties of kombucha black tea and chamomile with and without methylsulfonylmethane. Treatments were applied topically to denuded Balb/c mice and observed for hair growth initiation time, density and length in 15 and 30 days in addition to microbiological identification of the main yeast and bacteria. Results showed significantly better hair density and length in groups treated with kombucha black tea with and without MSM, followed by kombucha chamomile tea with and without MSM respectively. In addition, it has been found that MSM promotes hair growth significantly when combined with kombucha ferments. Microbiological analysis indicated the presence of *Zygosaccharomyces bailii* and *Acetobacter* genus in the tea broth. Black tea kombucha with MSM can be used as a natural alternative therapy for hair fall and a cost-effective treatment with fewer side effects compared to the synthetic drugs.

Keywords Kumbocha · Black tea · Chamomile · Hair growth · Zygosaccharomyces · Acetobacter

Introduction

Tea fungus or "Kombucha" is dated back to thousands of years in Manchuria, China and still widely-consumed in Europe and Russia. The traditional drink is considered as a probiotic with functional properties that has been reported to have many biological benefits upon drinking or topical application in different diseases such as arthritis, hypertension, cancer, AIDS, atherosclerosis, anorexia, hemorrhoids, indigestion. In addition, it possesses energizing, detoxification and hair re-growth properties observed personally and through testimonials, however, most of these claims are yet to be confirmed clinically (Ernst 2003; Sreeramulu et al. 2000). Kombucha is a symbiotic growth of acetic acid bacteria and yeast strains such as Schizosaccharomyces and Zygosacchromyces species cultured in a sugared tea in order to produce a sparkling apple cider taste after final fermentation by tea fungus.

The traditional way to prepare kombusha is by adding tea leaves to boiling water and allowed to infuse after which the leaves are removed. Sucrose is dissolved in the hot tea to provide the substrate for fermentation process along with the tea leaves. Vinegar or already prepared Kombucha is added to the mixture to increase its acidity with tightly closed container. The preparation is allowed to incubate at room temperature for one to two weeks, meanwhile, a new tea fungus is formed at the surface of the mixture and the fermented tea can be sieved and kept at 4 C to stop the fermentation process (Dufresne and Farnworth 2000). The combination of yeast-bacteria symbiosis produces different acids, xanthine, amino acids, sugars, elements such as Cu, Fe, Mn, Ni, and Zn; vitamin C, vitamin B complex and ethanol that are essential to provide biological benefits endowed by kombucha in addition to that prevent harmful organism's growth (Kapp and Sumner 2019). Also, the high level of glucuronic

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acid in the ferment avoid unexpected microbial contamination (Nguyen et al. 2015). Acetic acid bacteria convert glucose to gluconic acid and fructose into acetic acid. Caffeine and other xanthines of the tea stimulate the cellulose synthesis by the bacteria which float onto the surface of the mixture that form a suitable floated platform for the growth of both bacteria and yeast (Balentine 1997). Furthermore, yeasts metabolize fructose into ethanol and carbon dioxide, while ethanol in turn is oxidized to acetic acid by Acetobacter bacteria strains. Organic acids produced during the fermentation protects the colony from contamination with harmful foreign microorganisms (Sreeramulu et al. 2000).

The diversity of chemicals available in the kombucha drink that were previously mentioned in addition to polyphenols derived from tea leaves implies different pharmacological benefits such as hair re-growth properties. For instance, Hair follicles diameter and length were increased when NMRI mice skin injury treated with kombucha (Parivar, Yaghmaei and Heidari 2012). Many ways through which a drug might promote hair growth, such as; modifying the hair cycle, enlargement of the hair fiber, prolongation of the anagen phase or shortening the telogen phase, or a combination of these mechanisms. Infusions from other plants have been reported to be promising alternative to black tea as studies have indicated (Villarreal-Soto, Beaufort, Bouajila, Souchard, and Taillandier 2018). Therefore, the present research focuses on the assessment of the natural kombucha extract from black and chamomile tea as an alternative natural agent for management of hair loss.

Materials

Chemicals and equipment used are: minoxidil (Dar Al Dawa, Jordan), sugar (Nader, Jordan), chamomile flowers (Kabatilo, Jordan), pure black tea (Ceylon tea, Sri 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, 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.

Methods

Preparation of kombuch tea samples and microbiological evaluation

Four different preparations of komucha tea were prepared in accordance to previously described procedures (Jayabalan et al. 2007), with some modifications. All preparations, distinctive by different type of herbal tea and presence of MSM, were prepared under room temperature (25 ± 1) and protected from direct light exposure. Pure black tea (Ceylon tea, Sri 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. 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. Kombucha chamomile tea was prepared using the same procedure described above with chamomile flowers. Kombucha black tea and chamomile with MSM were prepared using the ratio of MSM: Kombucha black tea/chamomile were 5% w/v. Later, a sample from the extract was sent to the National Agricultural Research Center, Mafraq, Jordan to be analyzed for microbiological contents.

Selection and housing of experimental animals

A total of 48 adult male BALB/C mice were obtained from the animal house of Al-Isra University and were of the same age 7 weeks, to make sure that all hair follicles were synchronized in the telogen phase (Rho, Park, Hwang, Lee, and CD Kim 2005). The average weight was around (26–33 gm). The mice were provided with standard pellet diet and tap water ad libitum. All animal handling in the present study were conducted according to the guidelines of Institutional Animal Care and Use Committee (IACUC) and the research ethics committee of the faculty of pharmacy, Isra University has approved it under approval No. 55–5/2019–2020.

The mice were randomly distributed into eight groups ($n = 6$/group) and 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, Group 2 Positive control: Mice were treated with 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 kombucah black tea and MSM, Group 8: Mice were treated with a mixture of kombucha chamomile tea and MSM.

Assessment and evaluation of hair growth activity in mice

Following acclimatization period, an area of the hair (4 cm^2) from the dorsal part was shaved from each mouse using electrical razor and hair removal cream and then it was wiped with surgical spirit. Evaluation of hair growth activity was performed according to procedures describe previously (Park et al. 2015) as follows:

No change = 0 score.
< 30% darkening of the affected area = 1 score.
30–70% darkening of the affected area = 2 scores.
> 70% darkening of the affected area or hair growth in < 30% of the affected area = 3 scores.
> 70% darkening of the affected area and hair growth in 30–70% of the affected area = 4 scores.
> 70% darkening of the affected area and hair growth in > 70% of the affected area = 5 scores.
Hair growth in > 90% of the affected area = 6 scores.

Later on, the shaved area was then sprayed with 3 puffs (equals to 250µL) 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, length, and initiation time.

Statistical analysis

The results were presented as mean ± S.D. Data obtained was analyzed using the ANOVA followed by Tukey's test with $p < 0.05$ were considered statistically significant.

Results

Hair growth initiation time

The minimum time for the growth of hair from the denuded dorsal area was observed and the time taken for visible sign of hair was recorded in Table 1. The results undoubtedly indicated that treatment with kombucha/black tea with MSM (G7) reduced the time for hair growth initiation by 7 days, groups (G5, G6, and G8) reduced the time by 6 days, while the positive control group (G2) by 4 days compared to the negative control group, that suggests the beneficial effect of kombucha in promoting hair growth and pushing the hair cycle to enter the anagen phase quickly. Although some reduction in the time for initiation of hair re-growth was documented in group (G3, and 4) but it was not as noticeable as with kombucha treated groups.

Table 1 Time taken (in days) to initiate hair growth after starting respective treatments

GROUPS	Number of days for hair to initiate growth
G 7	7
G 5	8
G 8	8
G 6	8
G 2	10
G 3	12
G 4	12
G 1	14

Effect of different kombucha tea extracts on hair density

On days 15 and 30 of checking hair densities, it is so obvious that mice treated with kombucha/black tea with MSM and without MSM have gained the highest hair densities among all mice groups treated with other treatments. The results presented in Fig. 1 and supplementary Table 1 indicate that there is a significant enhancement in hair density ($p < 0.0001$) approximately 70%, 50%, 38%, 20% in groups G7, G5, G8, and G6 respectively in comparison to the negative control group (G1). The improvement in hair density was significant ($p < 0.0001$) for all treated mice after 30 days except with chamomile vehicle control group (G4). It is noticeable that there was a significant enhancement ($p < 0.001$) of hair density in mice treated with kombucha/black tea with MSM (G7), kombucha/chamomile with MSM (G8), and Kombucha black tea (G5) even when compared with the group treated with minoxidil (G2).

Effect of different kombucha tea extracts on hair length

The hair length analysis using caliper described in the method section on days 15 and 30 are shown in Fig. 2 and supplementary Table 2. The same trend of hair density can be applied here, mice treated with kombucha/black tea with MSM and without MSM (G7 and G5 respectively) have gained the highest hair length. The results indicated that there was a significant improvement ($P < 0.0001$) in hair length in all groups except with (G4) on both days; 15 and 30. The length of hair was tripled for G7 (9.3 ± 0.87,

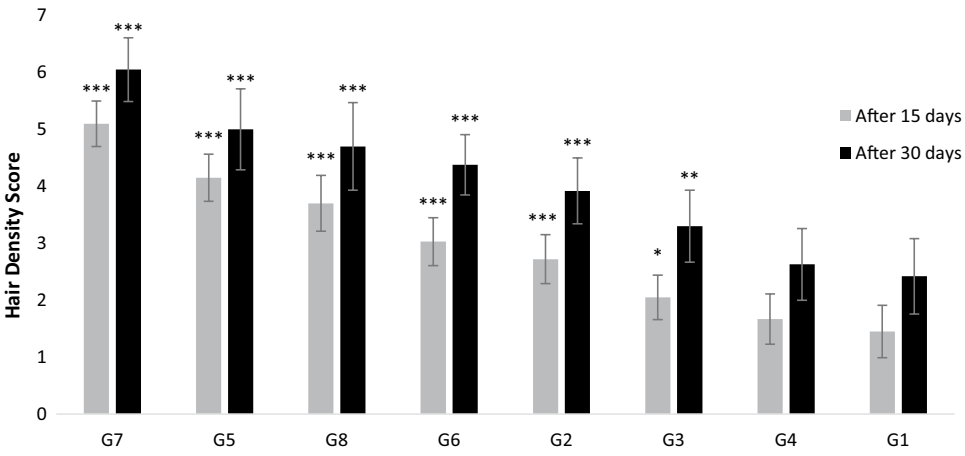

Fig. 1 Hair density activity based on the scoring system adopted from six mice in the treated mice group on day 15 and 30**.** G7 mice treated by kombucha/ black tea with MSM which have the best hair density scores followed by kombucha/ black tea, kombucha chamomile tea with MSM, kombucha chamomile tea, and minoxidil. Results are means ± SEM from six mice in each group, on day 15 and day 30. *$p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$ (p value was calculated compared to negative control group)

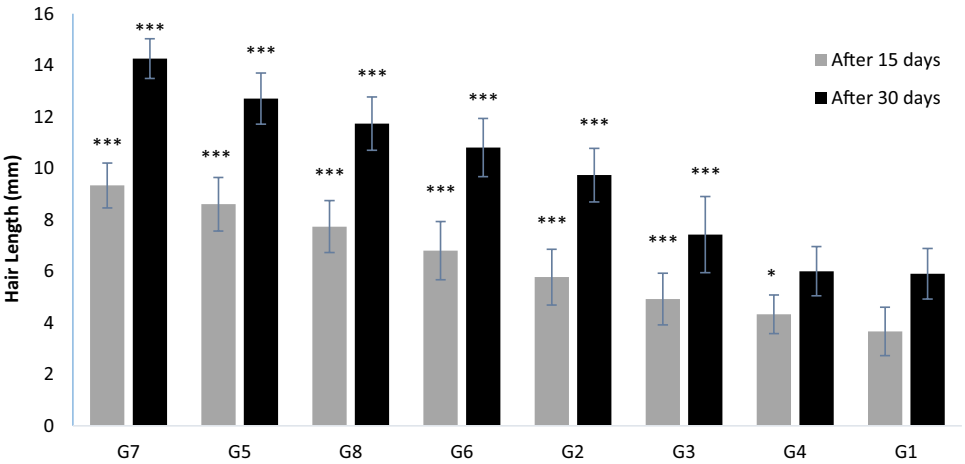

Fig. 2 Hair length in (mm) for G7 mice treated by kombucha/ black tea with MSM which have the best hair length results followed by kombucha/ black tea, kombucha chamomile tea with MSM, kombucha chamomile tea, and minoxidil. Results are means ± SEM from six mice in each group, on day 15 and day 30. *$p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$ (p value was calculated compared to negative control group)

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 days respectively, which shows that both kombucha and MSM had a pronounced effect on the length of the hair. Importantly, mice treated with black tea achieved a significant hair length growth, which was not observed with group treated with chamomile tea. It is also worth to mention that the length of hair for the treated mice with different kombucha extracts with or without MSM were significantly better ($p < 0.0001$) than minoxidil-treated group.

Microbiological analysis and hair regrowth scoring

The microbiological analysis revealed that Zygosaccharomyces bailii was the main fungus in the extract, while the acetic acid producing bacteria was from the Acetobacter genus. See supplementary resources. Differently, Fig. 3 represents the first day after removing hair from the dorsal part of the mice for all groups (Fig. 3: hair density score = 1). On day 15, we noticed that (G5 and G7) promoted the growth of hair in the shaved area by almost 70%, while (G 2, G6 and G8) by around 40% of the total denuded area with obvious longer hair in groups (G5, G6, G7, and G8) Fig. 4. However, the vehicle control groups (G3, and G4) especially the corresponding negative control group (G1) showed less noticeable, transient and irregular hair coats during this period. The whole denuded skin in the mice had been fully covered by hair when applied kombucha on day 30, however, groups (G5, and G6) still had small area with no hair and/or hair that is very short and not fully and densely covered Fig. 5. This obviously stipulates that kombucha extract with and without MSM promoted the re-growth of hair, with better result when combined with MSM.

Discussion

The results of this study demonstrated the potential effects of kombucha extract with MSM in denuded mice as a hair re-growth natural product. The presence of vitamins such as vitamins B, and C, minerals and amino acids depends on many factors such as symbiotic culture of yeast and bacteria,

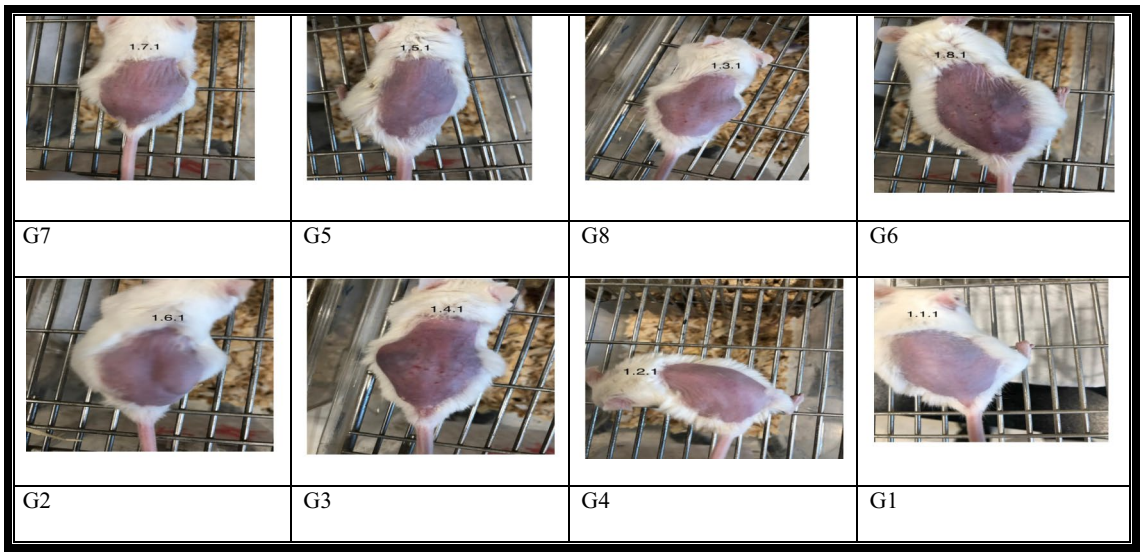

Fig. 3 A representative mouse of each group after on the 1st day of treatment. Each mouse was treated with 3 puffs of its respective treatments, G7: kombucah black tea and MSM, G5: kombucha black tea, G8: kombucha chamomile tea and MSM, G6: kombucha chamomile tea, G2: 2% minoxidil, G3: Black tea, G4: chamomile tea, G1: Negative control

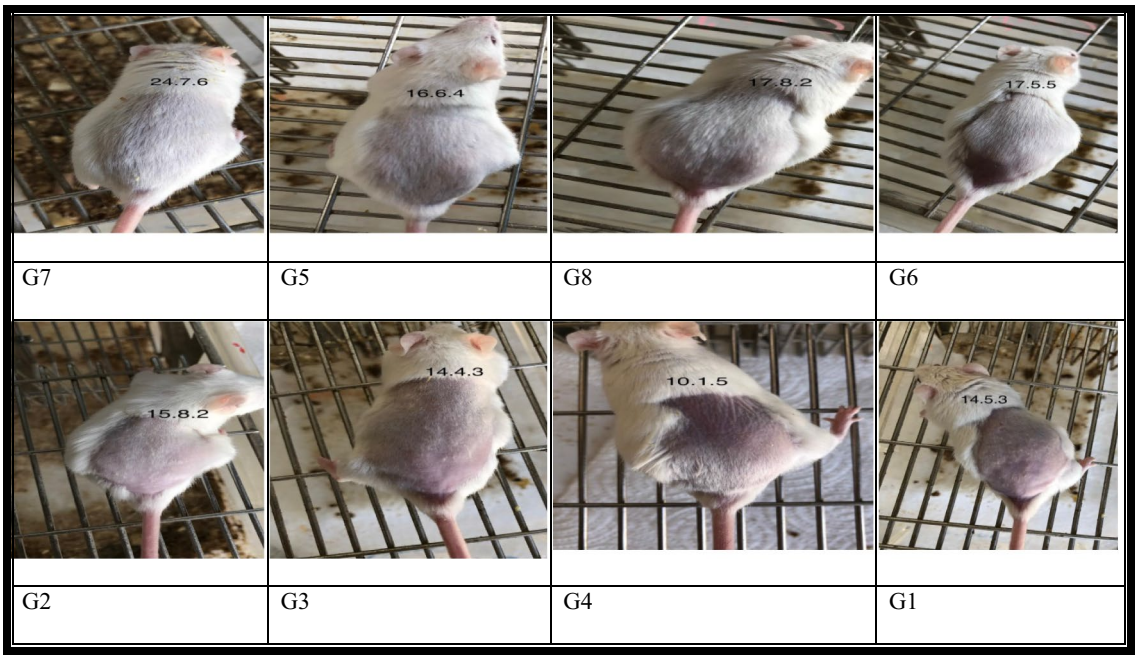

Fig.4 A representative mouse of each group after 15 days of treatment. Each mouse was treated with 3 puffs of its respective treatments, G7: kombucah black tea and MSM, G5: kombucha black tea, G8: kombucha chamomile tea and MSM, G6: kombucha chamomile tea, G2: 2% minoxidil, G3: Black tea, G4: chamomile tea, G1: Negative control

sucrose content, temperature, and type of tea used in the fermentation are all determinant factors for the components in the final product. The fermented kombucha produces plentiful of natural bioactive components, and their advantageous and beneficial application could speed up hair growth and regeneration. Kombucha is a symbiotic culture of number of yeast and bacteria, such as *Zygosaccharomyces bailii* that produces ethanol from the sugared tea (Karaman and Sagdic 2019), in addition to the *Acetobacter* bacteria that converts the ethanol to lactic and acetic acids (Martínez et al. 2018). Kombucha contains high levels of water-soluble vitamins, particularly; niacin, pyridoxine and ascorbic acid that

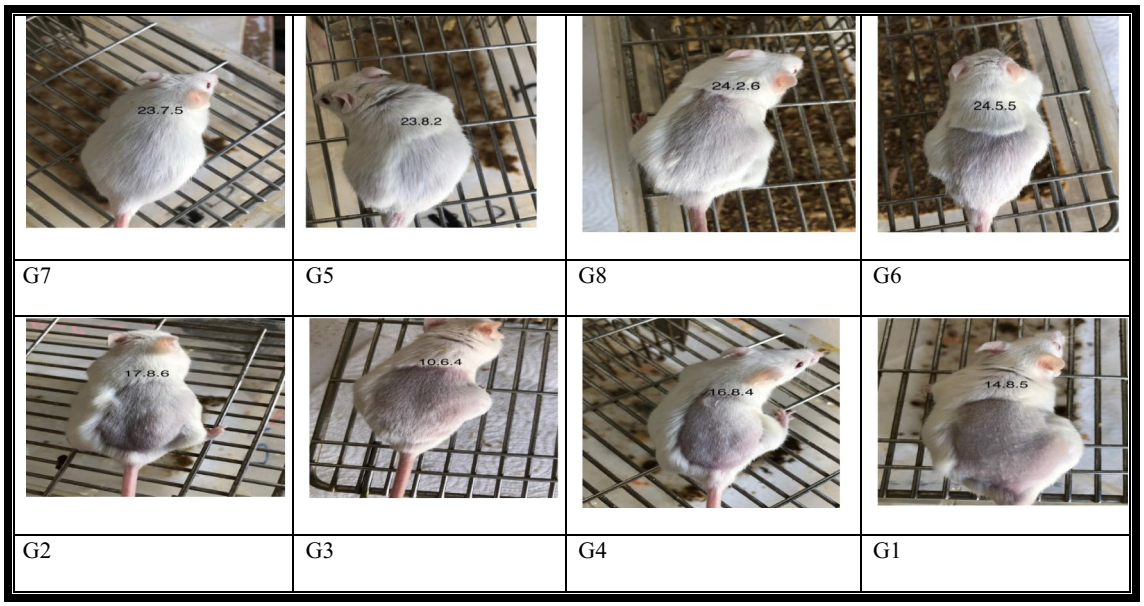

Fig. 5 A representative mouse of each group after 30 days of treatment. Each mouse was treated with 3 puffs of its respective treatments, G7: kombucah black tea and MSM, G5: kombucha black tea, G8: kombucha chamomile tea and MSM, G6: kombucha chamomile tea, G2: 2% minoxidil, G3: Black tea, G4: chamomile tea, G1: Negative control

function as antioxidant which reduces the oxidative stress and nourishes the dermal papilla cell thus reveals the ability of kombucha extract to enhance the growth of hair (DiBaise and Tarleton 2019). It was distinguished that the treatment with kombucha extract in this study resulted in expanding the length of the active phase "anagen phase" of hair cycle. In addition, the capability of kombucha extract to promote the growth of hair in denuded mice, was measured by different parameters, including hair initiation time, density and length.

The microbiological analysis in our study indicated the presence of *Zygosaccharomyces bailii* that produces organic acids from fermentation of sucrose into glucose and fructose and consequently it converted to lactic and acetic acid (both are alpha hydroxyl acid AHA) due to *Acetobacter* bacterial activities (Spedding 2015). AHAs are suggested to act as exfoliants which normalizes the keratinocyte accumulation or keratinization process on hair follicle that enhances the hair strand emerging through the scalp, which strengthen the hair shaft and prevent it from breaking off at the skin level (Rigdon and Packchanian 1974). Furthermore, AHAs are also 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 boosting in hair density (Woo et al. 2019).

Our results illustrate that kombucha fermented in black tea gave better results in hair promoting effects than when chamomile flowers with kombucha applied. This can be attributed to the presence of polyphenols in black tea that promoted hair growth such as caffeine and flavonoids. The amount of black tea major polyphenols which are thearubigins and theaflavins account for 12–18% and 3–6% of dry weight of black tea respectively (Halder et al.2005). Furthermore, the caffeine contents in black tea ranges from 2.79 to 2.93% and this is because many factors control it such as water temperature and extraction method (Horžić et al. 2009; Komes and Ganić 2009). It has been reported that caffeine inhibits phosphodiesterase, that in turn 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 dihydrotestosterone DHT on hair follicle and promotes the elongation of hair fiber (Bansal, Manchanda, and Pandey 2012; Fischer, Hipler, and Elsner 2007). Moreover, flavanoids of black tea such as EGCG have high affinity for estrogen alpha receptor and consequently they possess hair growth promoting activity (Hou et al. 2013).

Lastly, the addition of methylsulfonylmethane contributes in promoting hair growth, which is an organic sulfur-containing compound that occurs naturally in a variety of plants and animals including humans. 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). Additionally, some studies proposed that MSM forms bonds with hair follicle in order to delivering sulfur to the cortex layer of the hair, and thus promoting the conversion of telogen to anagen phase (Shanmugam et al. 2009).

Conclusion

From this work, it can be strongly proposed that kombucha fermentation of black tea has bioactive components that promote, restore and enhance the growth of hair through elongating the anagen phase of the mice model especially when combined with a penetrating agent such as MSM. This study recommends possible potential of kombucha extracts to be used as natural alternative therapy for hair loss. This natural alternative therapy is considered a cost-effective treatment and definitely has fewer side effects compared to the more expensive synthetic drugs.

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Compliance with ethical standards

Ethical statement All animal handling in the present study were conducted according to the guidelines of Institutional Animal Care and Use Committee (IACUC) and the research ethics committee of the faculty of pharmacy, Isra University has approved it under approval No. 55-5/2019-2020.

Conflicts of interest Mohammed Ayad Alboreadi has no conflict of interest. Manal Mamdouh Al-Najdawi has no conflict of interest. Qais Bashir Jarrar has no conflict of interest. Said Moshawih has no conflict of interest.

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