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Curcumin compounds and total microorganisms in turmeric kombucha as a potential therapy in rats obesity model

Zulfia Rosyidatun Nisa[1]*, Sinu Andhi Jusuf[2], Ratih Dewi Yudhani[3,4]

[1]Postgraduate Program of Nutrition Sciences, Universitas Sebelas Maret, Surakarta, 57126, Indonesia
[2]Department of Physiology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, 57126, Indonesia
[3]Department of Pharmacology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, 57126 Indonesia
[4]Biomedical Laboratory, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, 57126 Indonesia

**Correspondence*: zulfianisa_03@student.uns.ac.id

ABSTRAK

Latar Belakang: *Prevalensi obesitas meningkat signifikan dalam satu dekade terakhir dan berisiko menimbulkan berbagai komplikasi seperti diabetes melitus tipe 2, kardiovaskular, bahkan kanker. Terapi farmakologi obesitas seperti liraglutide dan orlistat dapat memberikan efek negatif pada tubuh seperti gangguan saluran pencernaan. Hal ini mendorong pengembangan terapi berbahan alami untuk obesitas. Kombucha dan kunyit kuning secara tunggal dilaporkan bersifat antioksidan dan mampu memperbaiki obesitas. Kombinasi kombucha kunyit kuning berpotensi dikembangkan sebagai minuman probiotik untuk obesitas karena senyawa bioaktif yang dikandungnya. Namun, eksplorasi fitokimia dan mikroorganisme pada turmeric kombucha masih sangat terbatas dan penelitian kombinasi kombucha kunyit kuning untuk obesitas belum pernah dilakukan sebelumnya.*
Tujuan: *Mengeksplorasi kandungan fitokimia dan total mikroorganisme kombucha kunyit kuning dan kemampuannya untuk memperbaiki obesitas.*
Metode: *Eksperimental laboratorik dengan rancangan pre-post control group dan deskriptif observasional. Analisis kurkumin menggunakan KLT Densitometri. Mikroorganisme dianalisis dengan metode pour plate kemudian dihitung berdasarkan total plate count. Tikus model obesitas diberikan tiga variasi dosis kombucha kunyit kuning selama 28 hari dan dilakukan penilaian perubahan indeks Lee yang diuji statistik menggunakan Kruskal Wallis ($p<0.05$) dan Post hoc Dunn Test.*
Hasil: *Hasil penelitian menunjukkan kandungan kurkumin pada kombucha kunyit kuning sebesar 0,73 mg/L dan total mikroorganisme sebesar 2,60 x 107 CFU/ml. Kombucha kunyit kuning memperbaiki indeks Lee tikus model obesitas dibanding kontrol negatif ($p<0.05$) dengan dosis paling efektif sebesar 4,4 ml/200 g/hari (dosis tertinggi di penelitian ini).*
Kesimpulan: *Kombucha kunyit kuning memiliki kandungan kurkumin dan manfaat mikoorganisme yang dapat memperbaiki indeks Lee pada tikus model obesitas.*

KATA KUNCI*:* *kombucha kunyit kuning; kurkumin; mikroorganisme; obesitas*

ABSTRACT

***Background**: The prevalence of obesity has increased significantly in the last decade, raising the risk of complications such as type 2 diabetes, cardiovascular diseases, and even cancer. Pharmacological therapies for obesity, such as liraglutide and orlistat, can have negative effects on the body, including gastrointestinal disorders. This drives interest in the development of natural-based therapies for obesity. Kombucha and turmeric individually are known for their antioxidant properties and their ability to address obesity. The combination of turmeric kombucha has the potential to be developed as a probiotic drink for obesity due to the bioactive compounds it contains. However, research into the phytochemical profile and microorganisms in turmeric kombucha is still limited, and no studies have been conducted on the combination of turmeric kombucha for obesity treatment.*

***Objectives:** To explore the phytochemical content and total microorganisms in turmeric kombucha and to evaluate its potential in improving obesity treatment.*

***Methods:** Laboratory experiments were conducted with pre-post control group design and descriptive observational analysis. Curcumin levels were analysed using KLT Densitometry, while microorganisms were identified through the pour plate method and quantified via total plate count. Obese model rats received three different doses of turmeric kombucha over 28 days, and changes in their Lee index value were assessed and statistically analysed using Kruskal Wallis ($p<0.05$) and Post hoc Dunn Test.*

***Results:** The findings revealed that the curcumin concentration in turmeric kombucha was 0.73 mg/L and the total microorganisms were 2,60 x 107 CFU/ml. Kombucha yellow turmeric improved the Lee index of obese model rat compared to negative control ($p<0.05$) with the most effective dose being 4.4 ml/200 g/day (the highest dose in this study).*

***Conclusions:** Turmeric kombucha contains curcumin and beneficial microorganisms, which can improve the Lee index in obese model rats*

KEYWORDS*:* *curcumin; obesity; microorganisms; turmeric kombucha*

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INTRODUCTION

Obesity is an abnormal condition characterized by increased fat accumulation in the body (1). According to the World Health Organization, 1 in 8 people in the world live with obesity, and the prevalence of obesity is around 890 million (2). In Indonesia, the prevalence of obesity reached 37.8%, indicating an increase in the incidence of obesity compared to 2018 of 35.4% (3). Individuals are categorized as obese for nutritional status if the body mass index (BMI) reaches >30 kg/m2 (2). The increase in body mass index is also due to a significant increase in body weight (4). Obesity is a major risk factor that can increase the incidence of cardiometabolic diseases such as cardiovascular diseases, diabetes mellitus, strokes, and some types of cancer (5, 6, 7). The implementation of current obesity can give pharmacological therapy such as orlistat and liraglutide (8). However, consumption of drugs can have negative effects on individuals, including gastrointestinal disorders such as diarrhea, nausea, vomiting, and swelling (9). Therefore, it encourages various research to develop an obesity natural therapy for lower side effects.

Kombucha is a sour and slightly sweet probiotic drink that originated in Northeast China (Manchuria) around 220 BC (10). It is traditionally made from a solution of black tea combined with a symbiotic culture of bacteria and yeast (SCOBY), which contains various types of lactic acid bacteria, acetic bacteria, and yeast fungi. Kombucha can be fermented for 10-14 days (11,12). In addition, kombucha has an antioxidant activity that is beneficial for health effects (13). Research states that the content of microorganisms ranges from 1 x 107 – 1 x 109 depending on the duration of fermentation (14).

Previous research has proven that kombucha can improve dysbiosis, type 2 diabetes mellitus (DMT2), fatty liver, and other diseases (1,15, 16, 17). Over time, kombucha has been made with different types of solutions, such as sea grapes kombucha, snake fruit kombucha, butterfly tea kombucha, and turmeric kombucha, which may offer enhanced health benefits (18, 19, 20). Turmeric (*Curcuma domestica Val*.) is a biopharmaceutical plant in the rhizome group with a distinctive yellow-orange color. Around 7,481.40 hectares of turmeric plants are grown across all provinces of Indonesia (21). The turmeric production in 2023 increased compared to the previous five years, with a gain of 206.65 thousand tons (22).

Turmeric has been proven to contain compounds such as antioxidants, curcumin, and essential oils. In China, turmeric is used in Traditional Chinese Medicine (TCM) for the treatment and prevention of cancer, diabetes, inflammation, and anti-obesity (23). Turmeric has been proven to contain curcumin compounds that can be useful as an anti-obesity and anti-inflammatory (24). The combination of probiotic fermentation between kombucha and turmeric is thought to increase the nutritional value of kombucha (25). Turmeric is used as a substitute for tea solutions and serves as the main ingredient in making kombucha. Research suggests that the fermentation process may enhance the bioavailability of curcumin in turmeric (26). However, limited studies have explored the effect of turmeric kombucha probiotics on obesity management. Therefore, this study aimed to determine the content of curcumin compounds and microorganisms from turmeric kombucha and its potential as an obesity therapy in obese rats model.

MATERIALS AND METHODS

Design and Materials

This study is a laboratory experiment research with a pre-post test control group design, while phytochemical compound research is descriptive and observational. This research obtained ethical clearance from the Faculty of Medicine, Muhammadiyah Surakarta University, with reference number 5159/A.1/KEPK-FKUMS/I/2024. The study focused on the content of curcumin compounds, the total of microorganisms consisting of lactic acid bacteria and Saccharomyces cerevisiae, and the effect of turmeric kombucha on the Lee index. The materials in this research were used to stater kombucha and turmeric powder. Starter and SCOBY Kombucha are obtained from the CV. Kebaikan Anugerah Alam, which has been certified Halal by MUI Indonesia (No. ID31110010237270723). The total microorganisms were examined at the FMIPA Biology Laboratory of Brawijaya University, Malang. Turmeric powder was obtained from PT. Phytochemindo Reksa, Cikeas, Bogor, West Java. The curcumin compound was examined in the Integrated Research and Testing Laboratory at Gadjah Mada University, Yogyakarta.

Animals Model

Male Wistar rats weighing 150-200 g were obtained from the House of Experimental Rats CFNS, Gadjah Mada University, Indonesia. Rats were divided into six groups of six rats each (n=6), totaling (n=36). The rats were placed in standard cages, with six rats per cage, and provided with a sterile cage. The box was kept in a bright, dry place with ventilation, and a room temperature which was maintained at 25 – 27̊C or 65 – 80̊F with a humidity of 75-90% (27). The rats were adapted for 7 days to ensure that no physical or physiological changes occurred before being given the treatment.

Obesity in the rats was induced using a high-fat high-carbohydrate diet (HFHFr) for 8 weeks. The production of HFHFr diet refers to the study of (28), with a composition of 32 g of AD II comfeed feed, 28 g of duck egg yolk, 40 g of beef fat, 12 g of chicken liver, and 4 g of butter which contains 610 kcal of energy, 56.64% fat, and 13.11% protein. The measurement of the Lee index was used as a parameter for the obese model of mice. The value of Lee index is obtained from the measurement of nasoanal weight and length, and then calculated based on the formula:

$$Lee\ index = \frac{Body\ weight\ (g)^{1/3}\ x\ 1000}{nasoanal\ length\ (cm)} \quad (1)$$

Rats are classified as obese models if they have a Lee index of 300 g/cm3 (29). In this study, five groups, other than the normal control, reached the Lee index of 300 g/cm3, thus confirming that the obesity model in this study was achieved, after which the intervention phase was conducted. The rats were given three variant doses of turmeric kombucha for 28 days. The groups were as follows: Group 1 (CN): Comfeed AD II (standard diet); Group 2 (C-): Obese rats + Comfeed AD II (standard diet); Group 3 (C+): Obese rats + orlistat 10.8 mg/200 g/day; Group 4 (T1): Obese rats + turmeric kombucha 1.1 mL/200 g/day; Group 5 (T2): Obese rats + turmeric kombucha 2.2 mL/200 g/day; Group 6 (T3): Obese rats + turmeric kombucha 4.4 mL/200 g/day. The rats were given turmeric kombucha orally using a gastric tube.

Turmeric Kombucha Preparation

The starter of kombucha was the kombucha that had been fermented for >14 days. All equipment used to produce turmeric kombucha was sterilized in boiling water at 100°C for five minutes to avoid unwanted bacterial contamination. Personal protective equipment and gloves are also used during the preparation. Here are the steps in the process of making turmeric kombucha: Ten (10) grams of turmeric powder is added to 1000 ml of boiling water for 5 minutes. After that, 10% sugar is added to the solution and stirred until homogeneous. The sweetened turmeric solution is placed in a sterilized jar and cooled at room temperature of 24-25°C. Starter kombucha 10% is mixed into the solution until homogeneous, then scoby gel is added and covered with a clean cloth. Turmeric kombucha fermented for 14 days. After the 14-day fermentation process, the turmeric kombucha glass container is refrigerated at a temperature <18^{o}C.

Microbial Determination

The quantity of microorganism colonies in turmeric kombucha was measured using the pour plate technique. This method is used to isolate and count living microorganisms in a liquid sample. The sample is mixed with a solid medium and incubated at 37°C for 24-48 hours (30). The visible colonies are then counted as CFU/mL using the following formula:

$$\frac{\mathrm{CFU}}{\mathrm{ml}} = \frac{Total\ number\ of\ colonies\ obtained\ x\ dilution\ factor}{Volume\ of\ specimen\ used\ (aliquout)} \qquad (2)$$

Previous studies have demonstrated that kombucha contains colonies of microorganisms consisting of lactic acid bacteria and the yeast fungus Saccharomyces cerevisiae (31). In this test, Previous research has shown that kombucha contains a colony of microorganisms consisting of lactic acid bacteria and the yeast Saccharomyces cerevisiae. In this study, De Mann Regosa and Sharpe (MRS) agar were used for isolating lactic acid bacteria, and Yeast extract Peptone Glucose Agar (YPGA) was used for Saccharomyces cerevisiae. The type of microorganism in this study consists of the lactic acid bacteria and the yeast fungus Saccharomyces cerevisiae. Pour plate is more suitable for isolating anaerobic and facultative microorganisms than any other method (32).

Phytochemical Determination

Curcumin testing with TLC-Densitometry KLT is an efficient, fast, accurate, and relatively cheaper method compared to other methods. Homogenize the turmeric kombucha and 25 mL of the samples in a porcelain cup. Dry in the oven at a temperature of 50oC for 24 hours. Then add 1 mL of ethanol and put it into a jar. Rinse the cup with 1 ml of ethanol Volumetric Flask. After that, add ethanol until the line boundaries. Next, spot a sample of 1 µL on a silica gel plate 60 F254 with a micro syringe, including standard curcumin regression. Put the plate into a chamber saturated with the toluene motion phase: glacial acetate acid (8-2). Lastly, curcumin levels are analyzed with the curcumin spot density at a wavelength of 425 nm. The curcumin test was done with two repetitions, and then the final result of curcumin content was based on the average of the two results from the analyses (33,34).

Statistical Analysis

The statistical analysis in this research was using SPSS (version 26, SPSS Inc). Statistical data analysis was performed using Kruskal Wallis (p<0,05) because, based on the analysis data, it was not normally distributed and homogeneous. The data were further analyzed using the post-hoc

Dunn Test to assess statistical differences between the groups.

RESULTS AND DISCUSSIONS

Characteristic of Materials

The turmeric kombucha used in this study was fermented for 14 days at room temperature. The yellow colour in turmeric kombucha is derived from turmeric powder, as illustrated in **Figure 1**.

Figure 1. Turmeric kombucha

Additionally, turmeric kombucha has a sour, slightly sweet, and fizzy taste. After the 14-day fermentation, the kombucha was transferred to a 250 mL glass bottle and stored in the refrigerator to maintain quality and extend its shelf life.

Analyses of Curcumin and Microorganisms in Turmeric Kombucha

This study found that turmeric kombucha contains 0.73 mg/L of curcumin. Another study demonstrated that turmeric kombucha contains 308.3 g/mol of curcumin, which acts as an anti-inflammatory and antioxidant to protect liver function in diethylnitrosamine-induced mice (35). Research on the curcumin content in turmeric kombucha is still limited.

Table 1. Curcumin and microorganisms in turmeric kombucha

Parameter	Unit	Result
Curcumin	mg/L	0.73 ± 0.04
Total microorganism cells	CFU/ml	2.60×10^7

The total content of microorganism cells consists of lactic acid bacteria and Saccharomyces cerevisiae. Based on the total plate count test, the results were obtained that these two types of microorganisms grew in turmeric kombucha 2,60 x 107 CFU/ml. The results of this study prove that turmeric kombucha contains a total microbiota of 2,60 x 107 CFU/ml, a result that is higher compared to previous research (2,0 x 107 CFU/ml) (36).

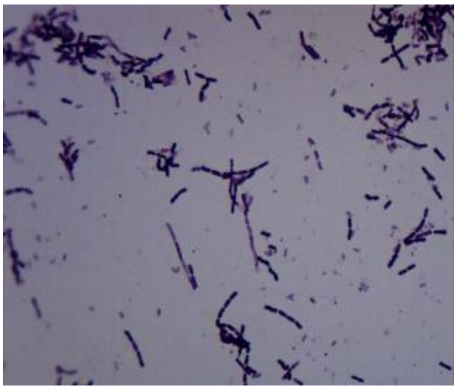


Figure 2

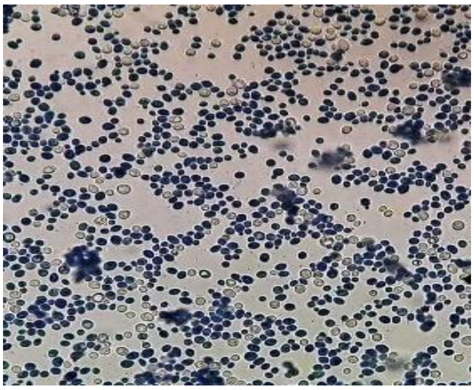
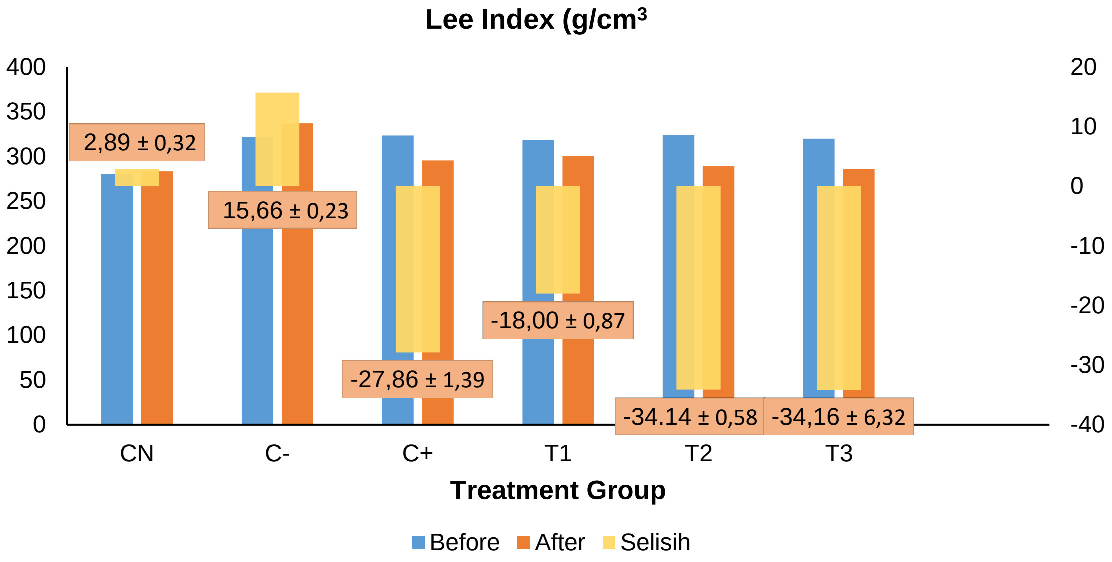


Figure 3

Description: Figure 2. Lactic acid bacteria cells and Figure 3. *Saccharomyces cerevisiae* yeast in Turmeric Kombucha. Observations are made under a microscope with a magnification of 400x.



(CN): Normal rats; C(-): obese rats; C(+): obese rats + orlistat; T(1): obese rats + turmeric kombucha 1,1 ml/200g/day; T(2) obese rats + turmeric kombucha 2,2 mL/200g/day; T(3) 4,4 mL/200g/day.
^{x}p < 0,05 vs CN *(Kruskal Wallis and Post Hoc Dunn test)*
^{y}p < 0,05 vs C- *(Kruskal Wallis and Post Hoc Dunn Test)*
^{z}p < 0,05 vs C+ *(Kruskal Wallis and Post Hoc Dunn Test)*

Figure 4. Graphic average of Lee index before and after treatment of turmeric kombucha

However, when compared to kombucha tea (4 x 109 CFU/ml), the content of turmeric microorganisms in kombucha is lower (37). A picture of the total microorganisms in the turmeric kombucha can be seen in **Figure 2**. A picture of the total microorganisms in the turmeric kombucha can be seen in **Figure 2** and **3**.

Lee index

The Lee index is an indicator of obesity in obese rat models. The Lee index value is calculated from measurements of body weight and nasoanal length. Rats are classified as obese if their Lee index is >300 g/cm³(29,38). Lee index measurements were performed before and after the intervention, this aimed to evaluate the effect of yellow turmeric kombucha on the Lee index values in obesity models of rats and the Lee index value shown in **Figure 4**.

The results show a significant decrease in the Lee index across all groups (p < 0.05) based on the Kruskal-Wallis statistical test Furthermore, the treatment groups given turmeric kombucha had lower Lee index values compared to the C- group. All doses of turmeric kombucha showed a decrease in the Lee index, with the highest reduction observed in the third dose (Δ = -34.16 ± 6.32). The decline in the value of the Lee index in the T2 and T3 groups was comparable to that of the C+ group (p=1,000). These findings align with prior research (39) where kombucha tea at 2.5 mL/100g was shown to reduce the Lee index in a fatty liver disease rat model. Another study found that sea grape kombucha reduces body weight in obese mice, which correlates positively with a decrease in the Lee index (18).

The Role Mechanism of Turmeric Kombucha Improves Lee Index

This study showed that turmeric kombucha contains curcumin 0,73 mg/L, and microorganisms 2,60 x 107 CFU/ml decreased the Lee index in the obese rats model (p<0.05). This study is in line with prior research (40,41) , showing that probiotics can improve obesity with decreased body weight and body mass index with

Short Chain Fatty Acids (SCFA). On the other hand, Curcumin also plays an important role in obesity therapy (24). The role of turmeric kombucha as a probiotic and the content of curcumin compounds can correct obesity through two mechanisms: improvement of dysbiosis and inhibition of adiposity differentiation through peroxisome proliferator-activated gamma receptor (PPAR-γ) (42, 43, 44).

In the human digestive tract, billions of microorganisms play a role in various functions. The interaction between the microbiota and the host can help in the process of decomposition and absorption of macronutrients and micronutrients, including enhancing immune function (45). In the intestinal tract, good bacteria like Bifidobacterium, and Lactobacillus grow side by side with pathogenic bacteria such as Firmicutes, including Clostridium in the intestines. The occurrence of obesity affects changes in the composition of the microbiota in the intestine. Obese individuals experience an imbalance in the number of bacteria called dysbiosis (46). Dysbiosis causes a decrease in both bacteria such as Bifidobacterium, Bacteroides, Lactobacillus, and Akkermansia, and an increase in Firmicutes, Prevotella, and Methanobrevibacter, which can affect the optimal production of SCFA (47,48). The condition of dysbiosis can also affect the increased risk of obesity in normal individuals because, at a time when SCFA production is reduced, it can increase the hormone ghrelin produced in the intestines so that increased hunger occurs (48). Probiotics and curcumin compounds benefit digestive health (49,50). The microorganisms in turmeric kombucha include lactic acid bacteria and yeast (2.60 x 107 CFU/mL), surpassing findings in earlier studies (36). Probiotics play a role in gastrointestinal homeostasis by increasing intestinal microbiota that correlates with optimal gut-brain axis function (GBA). Research on the relationship between obesity and GBA has been extensive, proving that GBA is linked to the regulation of certain hormones such as ghrelin, leptin, and glucagon-like peptide (GLP-1) through the vagus nerve (51). Obesity and dysbiosis lead to increased secretion of the hormone ghrelin and leptin resistance, which causes satiety signal disorders (52). Probiotics, rich in lactic acid bacteria, enhance SCFA production to signal satiety, thus controlling food intake (53). Furthermore, Lee index value improvement is also affected by the presence of curcumin compounds. Studies have indicated that curcumin supplementation has a positive role in reducing weight and body mass index in obese patients (44). The curcumin compound acts as an anti-obesity agent by inhibiting the early stages of adiposity differentiation through the suppression of the peroxisome proliferator-activated gamma receptor transcription factor (PPAR-γ) and increasing the monophosphate-activated protein kinase (AMPK) to lipolysis (54). Together, turmeric kombucha's probiotics and curcumin work synergistically to improve the Lee index in this study.

LIMITATION

The primary limitation of this study is its focus solely on curcumin, without examining other phytochemicals or specific types of lactic acid bacteria in turmeric kombucha.

CONCLUSIONS AND RECOMMENDATIONS

Turmeric kombucha, which contains curcumin and microbiota, has shown potential to improve obesity conditions, as indicated by reductions in the Lee index in obese rat models. Further research is expected to explore other phytochemicals in turmeric kombucha and analyse specific types of lactic acid bacteria, along with their effects on other markers of obesity.

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