

Research Article

Evaluation of Heavy Metal and Microbial Contamination in Green Tea and Herbal Tea Used for Weight Loss in the Palestinian Market

Murad N. Abualhasan ¹, Nidal jaradat ¹, Mohammed Hawash,¹ Rama Khayat,¹ Eman Khatatbeh,¹ Malak Ehmidan,¹ and Munir Al-Atrash²

¹Faculty of Medicine and Health Sciences, Department of Pharmacy, An-Najah National University, Nablus, State of Palestine

²Jerusalem Pharmaceutical Company, Quality Control Department, Ramallah, State of Palestine

Correspondence should be addressed to Murad N. Abualhasan; m_abualhasan@najah.edu

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The use of green tea and herbal tea for weight loss is increasing worldwide owing to the rising rates of obesity. There are concerns about the safety and quality of these herbal products owing to their increased consumption worldwide. In this study, we evaluated randomly collected samples of green tea and herbal tea and tested them for heavy metal and microbial contamination. Eighteen samples of green tea or herbal tea of widely used brands in Palestine were tested for heavy metal and microbial contamination. The results showed that 7 of the samples had toxic heavy metals such as chromium (Cr) and lead (Pb), and their concentrations were above the allowable limits set by the World Health Organization (WHO). Moreover, 6 of the samples that were tested had microbial contamination with high total aerobic microbial count (TAMC) and total yeast and mold count (TYMC). This could be due to improper handling and storage conditions of these herbal products. This study is the first of its kind in Palestine, and its results are forewarning to all the responsible authorities, including the Ministry of Health (MoH), to take immediate corrective actions such as quality control testing, auditing, and registration of marketed tea products.

1. Introduction

The use of green tea and herbal tea for weight loss has been increasing worldwide, concomitantly with the rates of obesity [1, 2]. These products are easily accessible, trusted, easy to use, inexpensive, and thought to be safer compared to conventional medications. These factors led to an increased use of these types of teas [3, 4]. Globally, tea is the second most consumed drink after water [5]. Three billion kilograms of tea are produced each year worldwide [6]. Nearly 20%–22% of the total tea consumption is of green tea, primarily in China, Japan, Korea, Morocco, and few other countries in North Africa and the Middle East [5, 7, 8].

Green tea is “nonfermented” and obtained from *Camellia sinensis*, whereas other herbal teas used for weight loss are from several other tea plants, which are sometimes used

in combination with green tea. These include *Pimpinella anisum*, *Mangifera indica*, *Garcinia gummi-gutta*, *Terminalia bellirica*, *Aloysia citrodora*, *Matricaria chamomilla*, *Centaurea cyanus*, *Urtica*, *Eucalyptus globulus*, *Plantago ovata*, *Laurus nobilis*, *Tilia europaea*, *Cymbopogon citratus*, *Mentha spicata*, *Rosa canina*, *Echinacea angustifolia*, *Terminalia chebula*, *Vaccinium oxycoccos*, *Cuminum cyminum*, *Zingiber officinale*, *Salvia officinalis*, *Cinnamomum verum*, *Foeniculum vulgare*, *Portulaca oleracea*, and *Senna italica* [5, 9].

Huge concerns about the safety and quality of herbal products have been rising. This is due to the rapid and widespread increase in the consumption of herbal products over the last few years [10]. The level of heavy metal contamination in herbal products is recommended to be minimal because these consequently add to the level that has

already been consumed through daily diet [10]. The allowable limits of heavy metals depend on the nature adjusted according to regional or national variations [10]. The allowable limits of selected heavy metals in edible medicinal plants, according to international guidelines, are provided in Table 1.

Microbial contamination in herbal products may occur at any stage of product preparation, from planting and harvesting to packaging, distribution, and storage. The presence of microorganisms at certain levels may indicate poor quality of such products [11]. The allowable limits of microbial contamination vary according to the type of herb and its intended use [10]. The allowable limits of microbial contamination in herbal products recommended by the WHO are the total aerobic microbial count (TAMC) must not exceed 10^7 CFU/g; the total yeast and mold count (TYMC) must not exceed 10^5 CFU/g; and these products must be free from *Clostridium difficile*, *Salmonella*, and *Shigella*.

Currently, there is an inclination towards widely using different types of herbal products as complementary and alternative medicine in Palestine, owing to the biodiversity in this area [12]. Herbal products in the Palestinian market are regulated and controlled by the Ministry of Health (MoH) through the General Administration of Pharmacy by the Drug Registration Department. This department is responsible for the registration of imported herbal products and food supplements. The affiliated quality control department is responsible for quality testing and implementing the rules of Good Manufacturing Procedure (GMP) for medicinal herbs packed in Palestinian establishments. The laws and regulations regarding herbal products have been recently established and updated. The MoH has the right to withdraw any herbal products that prove to have a dangerous side effect. The recommendations of the MoH on the allowable limits of heavy metals in herbal medicine are based on the recommendations of the WHO and Food and Agriculture Organization (FAO) [13–15]. However, there is still a gap in implementing these rules and regulations with regular quality testing for microbial and heavy metal contamination in green tea and herbal tea that are sold in the Palestinian market [16].

Several studies were carried out globally to evaluate the contamination in herbal products. These were conducted in countries, such as Pakistan, Tanzania [17–20], Italy [21], Ghana [22], Nigeria [23], Brazil [24], South Africa [25] and some Arab countries, such as Palestine, Saudi Arabia, the United Arab Emirates, and Egypt [26–30]. The main objective of these studies was to detect the presence of microbial and/or heavy metal contamination, such as lead (Pb), cadmium (Cd), manganese (Mn), iron (Fe), chromium (Cr), zinc (Zn), mercury (Hg), nickel (Ni), and arsenic (As) in some common medicinal plants and types of teas and to assess their safety according to international limits.

The results of these studies showed that manganese and cobalt exceeded the allowable limits [31]. Another study in Tanzania showed that the concentrations of Mg, Mn, Fe, and Ni in medicinal plants were higher than the allowed limits [32]. It is important to emphasize that even if the heavy

metal contamination is within the internationally accepted limits, there is still a high chance of accumulation of these elements in the body that reach toxic levels [33]. In the Middle East, the results of several studies showed that the heavy metals, such as Pb, Cd, Cu, and Zn in the herbal samples that were tested, were above the allowable limits. These results indicate that long-term use of contaminated herbal products may pose a potential health risk to consumers [23, 34, 35]. A comparative assessment of the quality of commercial black and green tea was conducted in Italy to check microbial and fungal contamination (total bacterial count, fungi, *Escherichia coli*, *Pseudomonas* spp., and *Clostridium perfringens*). The study showed that there was no microbial contamination that could be harmful to consumers, except for the contamination by mycotoxins such as ochratoxin A, which was above the allowable limits in food products in 50% of analyzed samples [36].

The objective of our study was to perform a quality check for the microbial and heavy metal contamination in green tea and herbal tea that claims to aid weight loss and is widely used among the Palestinian population. The results of the study reflected the actual situation of this problem in Palestine and will help raise the issue of contamination in herbal products to the concerned authorities.

2. Materials and Methods

2.1. Chemicals and Reagents. The following reagents were used throughout this study: tryptic soy agar (Difco Laboratories, Detroit, USA), Sabouraud dextrose agar (Difco™; France) (for preparing the medium), tryptic soy broth (Difco™; France), and Tween 80 (Difco™; France), which was used to prepare Fluid 3 for microbiological testing. Nitric acid (70%) (Riedel-de Haen, Seelze, Germany) and perchloric acid (70%–72%) (Riedel-de Haen, Seelze, Germany) were used for acid digestion of the herbal samples.

2.2. Instrumentation. An atomic absorption spectrometer (model: iCE 3000; Thermo Fisher Scientific) was used for the quantitative analysis of heavy metals. An autoclave (model: DLOV 3764; De Lama) was used for the sterilization of Fluid 3 (F3) and the agar mediums. A laminar air flow chamber (BBS-V1300; Biobase) and incubators (BC3100-R1; Biorold) were used for microbiological testing of the herbal samples, and Vortex Genie 2 was used for sample mixing.

2.3. Collection of Tea Samples. Eighteen samples were collected from either herbal medicine shops or community pharmacies. The samples were either green tea or herbal tea used for weight loss belonging to widely used brands in Palestine. Each tea sample was from a different brand, and the samples were either packed locally in Palestine or imported. The collected tea samples were primarily and widely used for weight loss.

All the samples that were collected were fine particles; thus, the samples did not require any further processing before experimentation.

TABLE 1: Examples of allowable limits of toxic heavy metals according to the World Health Organization (WHO).

Element	For edible plants (mg/kg)	For medicinal plants (mg/kg)
Nickel (Ni)	1.63	1.5
Copper (Cu)	3	10
Zinc (Zn)	27.4	50
Cadmium (Cd)	0.2	0.3
Lead (Pb)	0.43	10
Chromium (Cr)	0.02	1.3

They were stored in airtight glass containers in a dark, dry, and cool place during the period of the study.

2.4. Elemental Analysis

2.4.1. Acid Digestion of the Herbal Sample. The acid digest was prepared by oxidizing 0.2 g of the dry powder from tea bags, with 10 mL of an acid mixture of 2:1 ratio (nitric acid: perchloric acid), which was then stirred and kept overnight at room temperature. The mixture was then filtered, and 1 mL of the filtrate was diluted to 25 mL with distilled water. The diluted samples were used in the quantitative measurement of Pb, Cd, Cu, Ni, Cr, and Zn, using an atomic absorption spectrophotometer. These samples were prepared in triplicate for each tea brand, and each of those were tested three times; the average values and standard deviations were recorded.

2.4.2. Quantitative Elemental Analysis. For analysis, four standard concentrations of each heavy metal were prepared. The digested samples were diluted with water to be within the range of the calibration curve. The concentrations of the heavy metals in the samples were determined using the calibration curve and regression equation [37]. The four-point calibration of the concentration for each heavy metal that was studied is provided in Table 2.

2.5. Microbiological Testing. The tests for bacterial and fungal contamination were conducted according to the United States Pharmacopoeia (USP) [38]. Fluid 3 “F3,” a medium which was prepared by adding 15 g tryptic soy broth to 500 mL distilled water and 1 mL of Tween 80, was used to test for bacterial growth. Another medium was prepared by suspending 40 g of the powder of tryptic soy agar in 1 L of purified water. The mixture was thoroughly stirred with heat and boiled for 1 min to dissolve the powder completely.

Sabouraud dextrose agar was used to test for yeast and fungal growth. It was prepared by dissolving 65 g of the powder in 1 L of purified water, mixed thoroughly with heat, and frequently agitating to dissolve the powder completely. The agar medium was autoclaved at 121°C for 15 min. This medium was then used for the inoculation of fungi. The medium was placed in an oven at 50°C to maintain the liquid phase and was used whenever required.

Consequently, dry tea samples (3 g) were added to the corresponding preparations of Fluid 3 (30 g). The samples were diluted to prepare the following concentrations: 10, 10²,

10³, and 10⁴. Each dilution was inoculated on plates of tryptic soy agar and were incubated at 30–35°C for 48 h for bacterial identification. Fungal testing was performed by inoculating the prepared dilutions on plates of Sabouraud dextrose agar and incubating the plates at 20–25°C for 5 days. The results were reported as colony-forming units (CFU) per gram or mL.

2.6. Statistical Analysis. The Statistical Package for the Social Sciences software (SPSS) was used to perform descriptive statistical analysis. Analysis of variance (ANOVA) was used to test if there was a statistically significant difference between the average values. Pearson’s chi-squared test was used to determine whether there was a significant difference between the expected and observed frequencies of more than one category. In all the statistical tests, if the *p* value was less than 0.05, the null hypothesis was rejected, and it was concluded that a significant difference exists.

3. Results and Discussion

Products for weight loss that are made from natural sources are widely used by consumers because of their belief in its safety. Green tea is one of the most common natural ingredients in over-the-counter products for weight loss, which are available worldwide. A total of 18 samples of different brands of tea were analyzed for heavy metal and microbial contamination. The samples were either of green tea or herbal tea, which is frequently used by people who follow weight loss regimens. The majority of the tested samples were of green tea (60%), while other herbal teas constituted 39% of the tested samples. These percentages reflect the consumption by the public because a majority of the people think that green tea is safer as well as cheaper than other herbal teas for weight loss (Figure 1).

The tea samples were either locally packed or imported from other countries. Majority of the samples were imported from other countries (89%), while the rest (11%) were from local sources (Figure 1). The source of tea sample reflects the trends in selling herbal products in the local Palestinian market. The majority of herbal products, which are sold as weight loss products, are imported. There are only few local manufacturers of herbal medicine in Palestine; thus, their share in the local market is low compared to the imported products [39].

The results of the test for heavy metal contamination in the samples showed that all the samples had passed the test for Cu, Ni, and Cd and failed the test for Cr. Additionally, 3 of the samples had failed the test for Pb. The concentration of

TABLE 2: Calibration of concentration for each heavy metal.

Metals	Ni (ppm)	Cu (ppm)	Zn (ppm)	Cd (ppm)	Pb (ppm)	Cr (ppm)
Standard #1	0.05	0.5	0.5	0.5	1	0.5
Standard #2	0.1	1	1	1	2.5	1
Standard #3	0.5	2	2	2	5	2
Standard #4	1	3	3	3	10	3

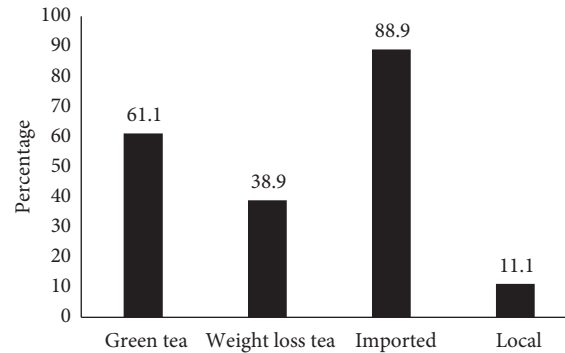


FIGURE 1: Source of tea samples.

TABLE 3: Concentration (ppm) of each heavy metal that was identified.

No.	Lead (Pb)	Nickel (Ni)	Chrome (Cr)	Zinc (Zn)	Copper (Cu)	Cadmium (Cd)
1	0.101 ± 0.0103 Pass	0.248 ± 0.0270 Pass	0.234 ± 0.0340 Fail	0.063 ± 0.0088 Pass	0.029 ± 0.0210 Pass	0.011 ± 0.0109 Pass
2	0.110 ± 0.0303 Pass	0.355 ± 0.0151 Pass	0.251 ± 0.0460 Fail	0.074 ± 0.002 Pass	0.042 ± 0.0359 Pass	0.012 ± 0.0015 Pass
3	0.111 ± 0.0101 Pass	0.363 ± 0.0010 Pass	0.295 ± 0.0247 Fail	0.118 ± 0.010 Pass	0.534 ± 0.8439 Pass	0.012 ± 0.0019 Pass
4	0.122 ± 0.0303 Pass	0.421 ± 0.0242 Pass	0.345 ± 0.0223 Fail	0.121 ± 0.0087 Pass	0.019 ± 0.0233 Pass	0.014 ± 0.0025 Pass
5	0.123 ± 0.0030 Pass	0.449 ± 0.0283 Pass	0.386 ± 0.0280 Fail	0.124 ± 0.0171 Pass	0.014 ± 0.0219 Pass	0.011 ± 0.0008 Pass
6	0.112 ± 0.0122 Pass	0.455 ± 0.0480 Pass	0.424 ± 0.0170 Fail	0.156 ± 0.0482 Pass	0.018 ± 0.0171 Pass	0.018 ± 0.0006 Pass
7	0.110 ± 0.0123 Pass	0.514 ± 0.0359 Pass	0.462 ± 0.003 Fail	0.111 ± 0.0011 Pass	0.033 ± 0.0241 Pass	0.019 ± 0.0023 Pass
8	0.134 ± 0.0332 Pass	0.487 ± 0.0034 Pass	0.473 ± 0.0156 Fail	0.117 ± 0.0050 Pass	0.033 ± 0.0297 Pass	0.028 ± 0.0026 Pass
9	0.123 ± 0.0112 Pass	0.539 ± 0.0342 Pass	0.495 ± 0.0073 Fail	0.159 ± 0.0567 Pass	0.027 ± 0.0332 Pass	0.021 ± 0.0041 Pass
10	0.110 ± 0.0101 Pass	0.564 ± 0.0270 Pass	0.522 ± 0.0055 Fail	0.137 ± 0.0134 Pass	0.039 ± 0.0364 Pass	0.019 ± 0.0021 Pass
11	0.385 ± 0.1104 Pass	0.555 ± 0.0409 Pass	0.505 ± 0.0131 Fail	0.134 ± 0.0042 Pass	0.034 ± 0.020 Pass	0.031 ± 0.0020 Pass
12	0.449 ± 0.0397 Fail	0.586 ± 0.0138 Pass	0.564 ± 0.0143 Fail	0.125 ± 0.0057 Pass	0.021 ± 0.0203 Pass	0.031 ± 0.0020 Pass
13	0.381 ± 0.0460 Pass	0.571 ± 0.0315 Pass	0.539 ± 0.0092 Fail	0.128 ± 0.0039 Pass	0.051 ± 0.0498 Pass	0.039 ± 0.0042 Pass
14	0.374 ± 0.0377 Pass	0.579 ± 0.0123 Pass	0.640 ± 0.0022 Fail	0.171 ± 0.0152 Pass	0.121 ± 0.0252 Pass	0.041 ± 0.0027 Pass
15	0.369 ± 0.0234 Pass	0.562 ± 0.032 Pass	0.560 ± 0.0188 Fail	0.132 ± 0.0072 Pass	0.037 ± 0.0370 Pass	0.045 ± 0.0059 Pass
16	0.476 ± 0.0403 Fail	0.623 ± 0.0107 Pass	0.614 ± 0.049 Fail	0.139 ± 0.0113 Pass	0.013 ± 0.0140 Pass	0.050 ± 0.0031 Pass
17	0.484 ± 0.0911 Fail	0.595 ± 0.0078 Pass	0.646 ± 0.0260 Fail	0.126 ± 0.0034 Pass	0.020 ± 0.0285 Pass	0.050 ± 0.0012 Pass
18	0.340 ± 0.0303 Pass	0.614 ± 0.0232 Pass	0.640 ± 0.0113 Fail	0.132 ± 0.0016 Pass	0.034 ± 0.0286 Pass	0.0473 ± 0.0044 Pass

each heavy metal that was identified in each sample are provided in Table 3.

The resulting concentration of Pb varied depending on the type of tea sample. Notably, all green tea samples had passed the test for Pb, while only 4 out of 7 samples of herbal tea had passed this test (Figure 2). Therefore, we performed a chi-squared test to determine the statistical difference, and the result showed significant difference ($p = 0.043$) between green tea and herbal tea. These results show that the lead contamination is less in green tea compared to herbal tea. Herbal products for weight loss consist of a mixture of many herbs that are cultivated from many sources; therefore, some of the content may contribute to high lead levels [40]. Moreover, most of the herbal products for weight loss that

are present in the Palestinian local market are not registered; thus, these items are not continuously monitored, and many of them could be adulterated and counterfeit brands [41].

Further statistical analysis was performed to determine whether the different source of the tea samples attributed to the lead contamination (Figure 3). A chi-squared test was performed to determine if the difference was statistically significant. The results clearly showed that there was no significance ($p = 0.66$). This indicates the quality of both types of teas, regardless of the source. As mentioned earlier, imported herbal products are not registered under the MoH, and many of them are contaminated due to improper handling, storage, and packing in local Palestinian establishments [42].

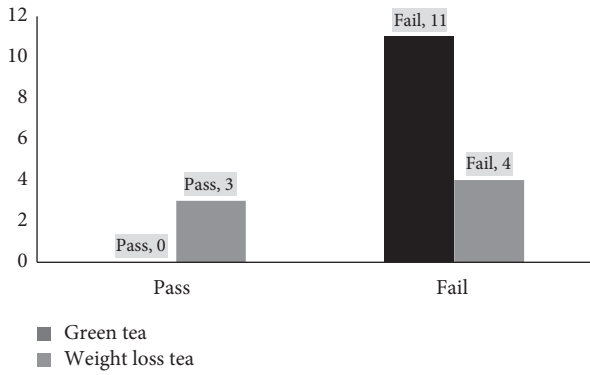


FIGURE 2: Concentration of lead in both types of tea samples.

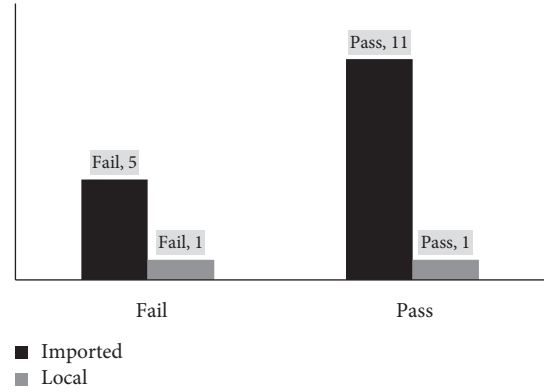


FIGURE 4: Results of microbial tests of the samples based on the source.

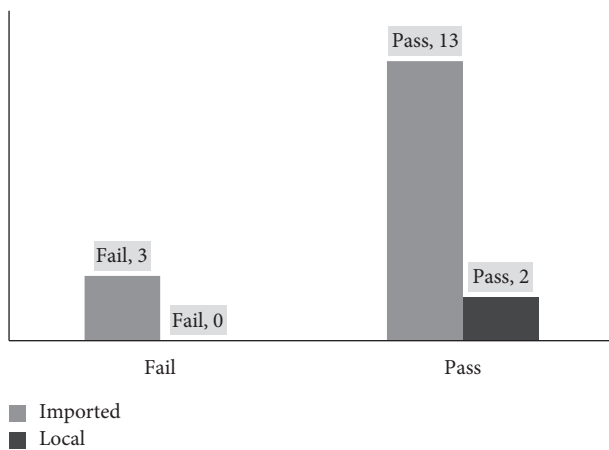


FIGURE 3: Concentrations of lead in the samples based on the source.

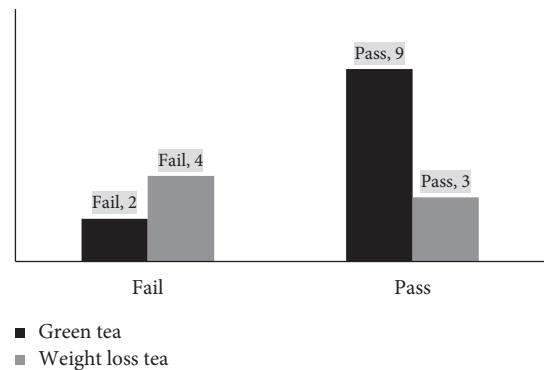


FIGURE 5: Results of microbial tests of both types of teas.

The results of the microbial tests showed that 33 % of the samples had microbial contamination.

The results of microbial tests from both sources of tea samples are shown in Figure 4. Six out of 18 samples had failed the test; 4 of the samples had a TYMC above the allowable limit (100 CFU/mL), and 1 sample had a TAMC above the allowable limit (1000 CFU/mL) [43, 44]. Moreover, 1 of the sample was contaminated with *E. coli*. The chi-squared test showed no significant difference in contamination between the imported and the local sources of tea samples ($p = 0.59$).

The results of the microbial tests of both types of teas are shown in Figure 5. The results showed that 2 out of the 11 green tea samples had failed the microbial test, and 4 out of the 7 samples were found to have microbial contamination. The results clearly show that the herbal tea was more contaminated than green tea. This could be because most of the brands of herbal tea for weight loss were not registered under the MoH; thus, they were not tested and did not undergo auditing. However, the chi-squared test showed no statistical significance ($p = 0.12$). Microbial contamination

in herbal products could be attributed to improper handling during dispensing, packaging, and/or nonadherence to good manufacturing practices [45, 46].

4. Conclusion

Green tea and weight loss herbal tea are widely used in the local and international market. Contaminated herbal tea proved to have detrimental and health hazards to its users specially when taken in large amount such as this types of widely used tea. This is the first study that evaluated the heavy metal and microbial contamination in green tea and herbal tea that are widely used for weight loss in Palestine. The results showed that many of the tested samples contain toxic heavy metals such as Cr and Pb, which were above the allowable limits. Moreover, majority of the tested samples had microbial contamination with *E. coli*, and some of the samples had TAMC and TYMC that were above the allowable limits. This study is a forewarning to all the responsible authorities, including the MoH, to take immediate corrective actions, such as quality control testing, auditing for proper storage conditions, and registration of marketed tea products.

Data Availability

No data were used to support this study

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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