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INTRODUCTION Coffee is one of the most consumed beverages worldwide and has a chief role in aiding the health benefits owing to its physiological effects on the central nervous and biological system. Coffee is known for its rich aroma, taste, and flavor¹. It comprises many different chemical structures and contains various substances like phenolic and alkaloids elements, vitamins, lipids, minerals, nitrogen compounds, chlorogenic acids, and carbohydrates². Drinking coffee is a common habit in developing and developed countries. According to the latest estimate from the International Coffee Organization (ICO), approximately 2.5 billion cups of coffee are intake in the world every day³.

Caffeine (1,3,7-trimethylpurine-2,6-dione) has known as an alkaloid that consists of two fused rings on associated purines⁴. Myriad people worldwide prefer coffee. Drinking coffee in constraint has several bioactive/volatile compounds, and it adds to the well-being of human health⁵. Effective use of caffeine consumption has reported better experimentation on human beings and animals. It gives ergonomics the ability to be physically active without a concurrent increase in the sensation of effort⁶.

There has been an increasing concern regarding Alzheimer's, Parkinson's, type 2 diabetes mellitus, cognitive functions, sleep quality, anxiety, and depression^{7,8}. Traditional medicine is common in developed and developing countries at the primary healthcare level. The World Health Organization (WHO) reported that people preferred traditional medicines at least 75-95% of the population in developing countries, and a majority of it involved the use of plant-based medicinal products or their active constituents^{9,10}.

Traditional Turkish coffee (TTC) is traditionally used in Turkey and most European countries. The preparation method of the TTC bean concentrates on fine powdering. The fine powder is then let to boil slowly in water (one spoon of coffee powder and one cup of water). The resultant half-boiled coffee solution is transferred to a cup and is left undisturbed while it settles at the bottom of the cup. Traditional Turkish coffee was found caffeine and other biologically active compounds in higher amounts when compared to other coffee preparation methods¹¹.

Traditional Turkish coffee plays a versatile role in traditional medicines like caffeine and other biological compounds for anti-cancer, anti-inflammatory, antidiabetic, antimicrobial, reducing the risk of mortality, cirrhosis, chronic and progressive diseases, coronary heart disease, Alzheimer's, and Parkinson's diseases^{12,13}. In this study, we present the preparation of TTC from Arabic coffee beans (ACB) and the effect of TTC consumption on healthy individuals. The ACB powder was characterized via UV-Vis spectrophotometer, Fourier transform infrared (FTIR) spectroscopy, scanning electron

microscope (SEM), and energy-dispersive X-ray spectroscopy (EDX) analysis.

The antimicrobial activity of ACB was evaluated against *Staphylococcus aureus* and *Escherichia coli*. The biocompatibility analysis of ACB was measured on human keratinocyte (HaCaT) cell line. The TTC consumption study on healthy individuals was conducted for 12 months. The medical history of individuals was evaluated. MATERIALS AND METHODS Materials Traditional Turkish Coffee bean was collected from a local supermarket, Izmir/Bornova, Turkey (Company Certificate Number: TR-34-K-004732), and the taxonomic position of the ACB was identified and authenticated.

The ACB was collected in large quantities, dried in the shade, and powdered. The HaCaT cell line was purchased from the National Centre for Cell Science (NCCS), Pune, India. Methods Physicochemical characterization The optical transmission/absorption spectra of ACB powder dispersed in water were recorded using a UV-Vis spectrophotometer (Hitachi, U3010) from 200 to 800 nm. Fourier transform infrared spectroscopy measurements were carried out to determine the formation and changes in the functional groups of ACB powder.

The spectra were measured at a resolution of 4 cm^{-1} in the frequency range of 4000–500 cm^{-1} using the Nicolet 360 FTIR Spectrometer. The surface morphology of the samples was visualized by SEM and EDX analysis (SEM Model LEICA stereo scan 440). Bacterial culture and antibacterial activity The antimicrobial activity was quantitatively evaluated against *S. aureus* (ATCC 25923) and *E. coli* (ATCC 11303). All the cultures were sub-cultured periodically and maintained on nutrient agar (NA) medium at room temperature ($30 \pm 2^\circ\text{C}$) for further experiments.

The antimicrobial activity effect of the prepared ACB powder sample was found by the modified agar well diffusion method. Muller–Hinton agars were sterilized and poured on Petri dish and allowed to solidify under laminar airflow. About 100 μL (108 CFU/mL (colony forming units)) of each bacterial culture was spread on the agar surface using a sterile glass spreader. Samples of 1 cm diameter were spread on the agar plate. The antibacterial activity was evaluated by measuring the diameter of the zone of inhibition against the organism. The control samples were prepared using amoxicillin and azithromycin antibacterial disc.

Biocompatibility analysis The cell line culture was maintained in Dulbecco's Modified Eagles Medium (DMEM) with 10% fetal bovine serum (FBS) at 37°C with 95% air and 5% CO_2 . Cell viability in terms of metabolic activity was evaluated calorimetrically by 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Cells (25×10^3 cells/mL) were seeded on ACB powder in a 24 well plate. After 30 minutes, a 10%

DMEM was added, and the cells were incubated for one and two days. The cell growth on the culture plate without ACB powder was taken as control. The percentage of cell viability was calculated compared to the control.

The cells were stained with acridine orange, and their morphology was viewed using fluorescent microscopy¹⁴. Preparation of Traditional Turkish Coffee The preparation of TTC was done according to Severini et al¹⁵. In brief, 7 g of coffee powder was taken and dispersed in 100 mL of cold water and stirred in the presence of sugar, having a homogeneous mixture before cooking. The resultant solutions were heated in a traditional coffee pot for three minutes at 100°C. Coffee was boiled to obtain foam, and cups in which coffee was served should also be hot.

Traditional Turkish Coffee assessment in healthy adults Ethical approval for this research was obtained via the local ethics committee-health science subcommittee of Ege Üniversitesi (EGE BAYEC) (with the following final decision and protocol number 19.03.2020/12-2020). The healthy adults were provided clear explanations about the study purpose, after which formal informed consent was obtained. The participants were given a written survey to establish their demographic information, such as age, education level, and coffee consumption.

Following primary data collection from the participants, anthropometric measurements such as body weight and height were gathered. The data for this sectional study was collected through face-to-face interviews. Participants were thrown out if they had a history of diabetic, metabolic, cardiovascular, and musculoskeletal disease or taking any other medicine, as per the medical history questionnaire. The effectiveness of this TTC was assessed on healthy adults with different age groups. Healthy individuals consisting of twelve men and twelve women aged 18-35 years were recruited to participate in this study (age 26.0 ± 2.3 y.o.; height 1.68 ± 0.04 m; body mass 74.2 ± 12.8 kg). Individuals were randomly divided into three groups depending upon the coffee used.

Group I (n=6) control, Group II (n=6) were individuals who used their regular instant coffee (RIC), Group III (n=6) were individuals who used TTC with sugar, and Group IV (n=6) were individuals who used TTC without sugar. All individuals gave informed consent for the study. The study was conducted for 12 months, and the medical history of individuals such as diabetics, coronary heart disease, and depression was also observed and evaluated. RESULTS AND DISCUSSION Ultraviolet-visible spectrophotometer The ultraviolet-visible spectrum of the ACB powder was shown in Figure 1. The strong interfering band was detected at wavelengths 210, 284, and 322 nm.

The composition of chlorogenic acid generates complexes with caffeine¹⁶. The important absorption peaks of chemical components are found in roasted coffee—the caffeine absorption peak at 281 nm and 320 nm due to the absorption of caffeic acid¹⁷. / Figure 1. UV-vis spectrum of ACB powder Fourier transform infrared spectroscopy The FTIR analysis was used to identify the functional groups of ACB powder (Figure 2). The description for each peak as a functional group in the FTIR spectra was mentioned. The absorption band at 1160-1370 cm⁻¹ and 1655-1745 cm⁻¹ were characteristics of the differences in the caffeine and chlorogenic acids. Spectra of organic molecules displayed 1020-1600 cm⁻¹, which could be a complex region of P-O, C-O, C-H, and C-N vibrations.

Flament¹⁸ has reported that roasted coffee flavor compounds form containing complex mixture like acid compounds, aldehyde, ketone, and ester plays a principal role in producing the fragrance of coffee. Nebesny and Budryn¹⁹ have demonstrated that coffee has potential antioxidants such as chlorogenic acid (phenolic compounds), caffeine, and hydroxycinnamic acids. / Figure 2. FTIR spectrum of ACB powder SEM and EDX analysis The SEM was used to observe the surface morphology of the prepared ACB powder (Figure 3). The average particle size of Turkish coffee powder was 508±7 mm, indicating its smooth surface and spherical shape.

The image observed the ACB powder surface a variety of flaky protuberances. Hao et al²⁰. reported that the morphology was analyzed as particle size, shape, and surface in the previous study. The EDX analysis results (Figure 4) of the ACB powder contained the minerals element such as CK, OK, MgK, KrL, PK, and AuM. / Figure 3. SEM of ACB powder / Figure 4. EDX of ACB powder Bacterial culture and antibacterial activity The ACB powder results in Figure 5 depict the antibacterial activity (minimum inhibitory concentration or MIC) against *S. aureus* and *E. coli*.

The ACB highest antibacterial activity (Table I), whereas control samples (amoxicillin and azithromycin) were the low zone of inhibition²¹. The antimicrobial effectiveness of ACB powder is due to the characteristics of the potentiality of caffeine²². Nuhu²³ has studied that the extraction of coffee powder of the bioactive compounds has a predominant function in antibacterial activity. Caffeine contains purine alkaloid compounds to DNA by inhibiting the incorporation of adenine and thymidine and inhibiting protein synthesis. Similar studies on *S. aureus* and *E.*

coli have resulted that caffeine inhibits protein synthesis, RNA impairs, and DNA synthesis²⁴. Caffeine is a natural antimicrobial activity, which growth of microbial strains reduction is nearly accomplice with changes in cell morphology in the various bacterial strains like *S. aureus* and *E. coli*²⁵. / a b Figure 5. Inhibition zone of ACB powder against *E. coli* (a) and *S. aureus* (b) Table I. Antimicrobial activity of ACB powder against *E. coli*

and *S. aureus* Organism _Inhibition zone (mm) _*E. coli* 3.96 ± 0.02 _*S. aureus* 3.97 ± 0.02 _ Biocompatibility analysis Examination of the biocompatibility of HaCaT cells in the uptake of ACB powder was analyzed using an MTT assay.

Figure 6 shows the results indicating the percentage of viable cells was found to be more in ACB powder when compared to control, which indicates the 100% biocompatibility of ACB powder to the HaCaT cells. ACB powder was evaluated through fluorescence microscopy, as shown in Figure 7. The previous study has proved the number of live cells after staining with a vital dye²⁶. Silverberg et al.²⁷ reported that fibroblast treated caffeine was protected toxicity, leading to more cell viability, enhanced cell morphology, mechanism, and effective antioxidant properties.

Early studies have obtained favorable results in cell viability and changes of morphologies with caffeine-treated HaCaT cells. Although cancer cell death by caffeine was investigated, caffeine is well recognized as a cancer cell death inducer, which helps anti-carcinogenic properties²⁸. / Figure 6. In vitro study of ACB powder on HaCaT cell line. The asterisks (*) indicate statistically significant differences compared to the control $p < 0.05$ / Figure 7.

Florescence micrographs (20X) of HaCaT cell line cultured on day 1 and 3 Traditional Turkish Coffee assessment in healthy adults The constancy and habits of three types of coffee, such as standard instant coffee, TTC with sugar, and TTC without sugar, were determined throughout 12 months. Each participant gave his or her informed consent to participate in the trial after hearing about all the procedures, risks, and benefits. The aroma and flavor of TTC could effectively add to the health benefits and help significantly reduce health risks (Figure 8).

The results of this study also comply with the statement, where the development of diseases like diabetes, heart disease, and depression was significantly less in group III and group IV compared to group II. The results of Morphett et al.²⁹ contradicts the above results, wherein they report the 50 years old female who used caffeine consumption (1-week) stage 1, caffeine ≤ 160 mg/day; (1-week) stage 2, caffeine ≤ 80 mg/day; (7-week) stage 2, caffeine ≤ 35 mg/day) had a significant reduction in behavioral change. It could also be stated that the antioxidant principles found in TTC might have also contributed towards oxidative stress prevention. Evaluation of coffee in the minimum number of people is an important study limitation.

Nevertheless, since the study results were promising, future studies are aimed to be conducted on a large scale. Alves and Oliveira³⁰ have reported that Turkey coffee consumption of about 1-2 cups per day may reduce the risk of colorectal cancer,

diabetes, Alzheimer's disease, Parkinson's disease, and heart disease. During the TTC trial, this study found significant increases in subjective measures of improved energy and trends in depression. It is essential to recognize the strengths and limitations of this study.

The research study plays a critical role in health psychology and stored details of individuals in context. During the experimental trial, all the Groups had an imbalanced blood pressure level. Excessive coffee drinking might also contribute to high blood pressure. Many studies have reported the effects of commonly consumed coffee beverages on blood pressure³¹. / Figure 8. Comparison of health benefits of control, RIC, TTC with sugar, and TTC without sugar
CONCLUSION The present study was carried out to analyze the properties of ACB powder.

The TTC prepared from ACB powder and consumed regularly may reduce the risk of diabetes, heart disease, and depression. In vitro analysis using HaCaT cell line proved the biocompatibility and its more viable cells present in ACB powder. The research study was checked for their efficacy as therapeutic Turkish coffee for different age groups of people. On the other hand, TTC appears to have more benefits, such as reduced acidity and high antioxidant activity, as well as no stomach issues.

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