Exploring of cardiac autonomic activity with heart rate variability in long-term kratom (*Mitragyna speciosa* Korth.) users: a preliminary study

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Background Kratom is a psychoactive plant used to enhance productivity among laborers in Southeast Asian countries. Previously, mitragynine, its major component, was demonstrated in vitro that might suggest possible risk of heart function abnormality. This study aimed to investigate the heart rate variability (HRV) indices of cardiac autonomic function in long-term kratom users (LKU).

Method A total number of 50 participants consisted of LKU (n=31) who regularly chewed fresh kratom leaves for at least two years and demographically matched control subjects (n=19). Resting electrocardiogram (ECG) signals were recorded from subjects for analyses of HRV indices in time, frequency, and nonlinear domains. The significant HRV indices between group differences were further classified using a linear discriminant model.

Results HRV analyses revealed a significant difference between groups in multivariate effect with a large effect size. Compared to control levels, LKU significantly increased RMSSD, HF, SD1, and SD1/SD2 and decreased LFn and LFn/HFn. The results indicated that LKU influenced cardiac autonomic function by changing sympathovagal balance that resulted in a predominant parasympathetic activity. The LFn/HFn and RMSSD were optimal classifications for discriminant LKU from controls with 76% accuracy. The present findings provided a possible precaution of kratom use on the autonomic cardiac function and the feasible application for detection of the autonomic cardiac function imbalance to prevent the progress of possible risks.

Exploring of Cardiac Autonomic Activity with Heart Rate Variability in Long-Term Kratom (*Mitragyna speciosa* Korth.) Chewers: A Preliminary Study

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Abstract

Background
Kratom is a psychoactive plant used to enhance productivity among laborers in Southeast Asian countries. Previous findings from in vitro research of mitragynine, a major component of kratom, suggested a possible risk of heart function abnormality. However, the cardiac autonomic function in long-term kratom users with chewing forms has never been studied. This study aimed to investigate heart rate variability (HRV) indices of cardiac autonomic function in long-term kratom chewers (LKC), compared to the control levels, and also to examine the correlation between HRV indices and relevant kratom use factors.

Method
A total number of 50 participants consisted of LKCs (n=31) who regularly chewed fresh kratom leaves for at least two years and demographically matched control subjects (n=19). Resting electrocardiogram (ECG) signals were recorded from subjects for three minutes to analyze the ultrashort HRV in the frequency domain. The normalized low frequency (LFn) and high frequency (HFn) were chosen to be the HRV indices to evaluate cardiac autonomic function. The

40   comparison of HRV indices between groups and the correlation between HRV indices and
41   duration and quantity of kratom use was further conducted in statistical analysis.
42
43   **Results**
44   The LKC significantly increased LFn together with enhanced HFn compared to the control group
45   tested, indicating that LKC changed cardiac autonomic function with parasympathetic
46   dominance. Furthermore, no significant correlation between the HRV indices and the duration
47   and quantity of kratom use was found, suggesting that the HRV indices were not relevant to
48   these factors. The present study provided scientific-based evidence of cardiac autonomic
49   modulation in long-term kratom chewers. LFn and HFn may be promising cardiac autonomic
50   indicators for monitoring health outcomes and possible risks in LKC.
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52
53   ## Introduction
54   Kratom or *Mitragyna speciosa* is a traditional herbal medicine in Southeast Asia,
55   particularly in rural areas of Thailand and Malaysia (Hassan et al., 2013). Due to its stimulant
56   and opioid-like effects, it was placed under schedule 5 of the Thai Narcotics Act, making it
57   illegal to sell or possess kratom in the past. Nowadays, the Thai government has delisted the
58   kratom plant as a narcotic to be partially legalized in the context of traditional and medical
59   purposes (Charoenratana, Anukul & Aramratwtana, 2021). However, the scientific evidence of
60   long-term kratom consumption associated health outcome in human remains elusive, particularly
61   in cardiac function.
62   Kratom typically promotes the dual effect depending on the dosage of use. The low dose
63   exerted a stimulant effect while the high dose presented an opioid-like effect (Prozialeck, Jivan
64   & Andurkar, 2012). According to pharmacological profiles, mitragynine and 7-
65   hydroxymitragynin are active components in kratom leaves extract (Veltri & Grundmann, 2019).
66   Both of them can act on opioid and non-opioid receptors. The later receptors, such as alpha-2
67   receptor, had effects on heart rate (Ellis et al., 2020). The effect of mitragynine on cardiac
68   function were investigated both *in vitro* and in human study. The molecular evidence of
69   cardiotoxicity from *in vitro* experiment was one of the major concerns about kratom effects.
70   Kratom was found to suppress potassium current (IKr) that might cause long QT syndrome,
71   resulting in lethal cardiac arrythmias (Tay et al., 2019). However, regular kratom users who
72   consumed kratom tea containing 434.28 mg of mitragynine on a daily did not appear to have
73   electrocardiogram (ECG) abnormalities with prolonged QT, except for a higher risk of sinus
74   tachycardia with a higher average heart rate measured at a resting baseline compared to non-
75   kratom using controls (Leong Abdullah et al., 2020). Regarding the evidence, regular users who
76   drink kratom tea appear to show sympathetic dominance.
77
78   In contrast to kratom tea consumption, the different form of long-term kratom
79   consumption was also found in older people who chewed kratom leaves in the traditional style
80   (Charoenratana, Anukul & Aramrattana, 2021). They usually chewed kratom leaves at very high
81   amounts (10-30 kratom leaves per day) to prolong and tolerate extreme fatigue (Henningfield,
82   Fant & Wang, 2018). With high quantities of consumption, they experienced relaxation and calm

83   (Johnson et al., 2020). It is possible that those LKC may have a predominant parasympathetic
84   activity. However, these effects of kratom consumption have never been directly confirmed with
85   measurable autonomic cardiac activity.

87   Heart rate variability (HRV) is a noninvasive and inexpensive method to detect the
88   autonomic cardiac function modulated by heart-brain interaction. It describes the oscillatory
89   patterns of time intervals between consecutive adjacent heart beats (electrocardiogram RR
90   intervals) (Shaffer & Ginsberg, 2017). High HRV exhibited in healthy subjects was found to
91   reflect the regulation of the ANS in adaptation to environmental and psychological challenges,
92   whereas low HRV was found in psychological and pathological problems (Maule et al., 2008)
93   (Henry, Minassian & Perry, 2012) (Ingjaldsson, Laberg & Thayer, 2003) (Barutcu et al., 2005).

95   In general, the gold standard of HRV measurement required 24-hour and 5-min periods
96   for long- and short-term ECG recording, respectively. The short-term HRV is more widely used
97   than the long-term HRV due to its practical process and data analysis. However, the standard
98   HRV evaluation is still long for routine healthcare purposes that might cause discomfort during
99   recording (Kim, Seok & Shin, 2021). In our preliminary study, local people found the ECG
100   recording uncomfortable and difficult to withstand. Therefore, ultra-short HRV (less than 5-min
101   ECG recording) is required to developed. Therefore, an ultra-short HRV (less than a 5-min ECG
102   recording) is required to develop. In the present study, the 3-min ultra-short HRV was chosen.

104   Two HRV indices in frequency domain analysis: low frequency (LF) and high frequency,
105   were usually used for ultra-short HRV analysis. HF component required a minimum recording
106   for 1 minute while LF component required at least 2 minutes (Malik et al., 1996). Values of the
107   HF component typically reflect parasympathetic activity (Shaffer & Ginsberg, 2017), while the
108   LF component is a more controversial interpretation. Some reports defined it as a sympathetic
109   activity, while others indicated the marker of both sympathetic and parasympathetic
110   modulations. To clearly interpret the index of sympathetic activity, the LF values needed to be
111   normalized by the total power. Since the total power is changed in the direction of LF and HF
112   when spectral components are computed in an absolute unit (milliseconds squared) (Papaioannou
113   Vasilios MD, et al., 2007). Moreover, the frequency components in the absolute unit produced
114   the power fluctuation. Therefore, the normalized LF (LFn) and HF (HFn) values are used to
115   attenuate the variation of power distribution (Vishwajeet, Singh & Deepak, 2020).

117   Therefore, the present study aimed to determine cardiac autonomic function assessed by
118   ultra-short HRV with LFn and HFn indices in LKC in comparison to non-kratom using controls.
119   Moreover, the relationship between HRV indices and kratom use variables, such as duration and
120   quantity of kratom use, was also investigated. The LKC group was hypothesized to show a
121   significant increase in HRV by enhancing the HFn and reducing the LFn indices of cardiac
122   autonomic function in LKC at rest, compared to the control levels. Moreover, the LFn and HFn
123   would be positively correlated with the kratom use variable.

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Materials & Methods
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Study design and participants

132   A final sample of 50 male subjects, consisting of LKCs (n=31) and non-kratom using
133 controls (n=19) were recruited from two local areas in southern Thailand (Bannasarn district,
134 Surat Thani province and Natawee district, Songkhla province). In these regions, kratom plants
135 have been widely used in cultural activities and daily life. The present study was conducted by
136 following the Declaration of Helsinki and the protocol was approved in writing by the Ethics
137 Committee of Prince of Songkla University, Thailand (HSc-HREC-63-017-1-1). The following
138 criteria were used to include kratom users: (a) age over 18 years; (b) daily kratom chewing (2
139 years); (c) last time chewing at least 2 hours to avoid the peak of kratom's acute effect (Singh et
140 al., 2019); (d) no history of psychiatric diseases as measured by the Brief Psychiatric Rating
141 Scale (BPRS); and (e) no history of illicit drug use and no underlying. In the cases of the control,
142 they had the same background as found in long-term kratom chewers, except their confirmation
143 that they did not use kratom at all. The LKC were recruited at first. After that, control subjects
144 were recruited to match the background variables of LKC. All participants provided verbal
145 informed consent to participate in the study. Finally, the participants were asked to complete the
146 questionnaires to collect the socio-demographic characteristics such as age, education, sex,
147 alcohol drinking, and kratom use history (e.g., duration or amount of kratom use per day).

148

ECG acquisition and HRV analysis

150   After participants completed the questionnaires, resting ECG signals were recorded
151 during an eye-closed period for 3 minutes. Although the standard short-term HRV measurement
152 was recommended for 5-minute recordings (Shaffer & Ginsberg, 2017), the ultra-short term
153 HRV features (less than 5 min) also showed good surrogate of HRV study (Castaldo et al.,
154 2019). The measurement of ECG signals was acquired using a standard limb lead I
155 configuration. The Ag/AgCl solid adhesive pre-gelled electrodes (Tian run, Beijing, China) were
156 fixed on the left and right sides of the clavicle with positive and negative electrodes,
157 respectively, with one grounded electrode attached to the participant's right ankle. These
158 positions were chosen to eliminate movement artifacts (Sarang & Telles, 2006). ECG signals
159 were recorded using BioAmp and Powerlab systems (ADInstruments, Castle Hill, Australia)
160 connected to LabChart software (version 7) at sampling rate of 1 k/s. The recorded ECG signals
161 were band-pass filtered at 0.3 -30 Hz according to previous studies (Baumert et al., 2007; Palma
162 et al., 2013, 2015).

163

164   The HRV analysis was performed by NeuroKit2: a Python toolbox for
165 neurophysiological signal processing (Makowski et al., 2021). Briefly, raw ECG signals were
166 then transferred to a laptop computer for storage and offline analysis. The ECG signals were first
167 cleaned by using *ecg_clean ()*. This function was also applied for artifact correction of the RR-
168 interval series by inputting the argument of *artifact_correction*. This argument was based on the
169 artifact detection algorithm as defined by the previous study (Lipponen & Tarvainen, 2019).
170 Then, HRV in the frequency domain was computed by Welch method with the *hrv_frequency ()*

171 function to extract the distribution of power spectrum density across the low-frequency (LF;
172 0.04-0.15 Hz) and high-frequency (HF; 0.15-0.4 Hz). The normalized low-frequency (LFn) and
173 high frequency (HFn) components that are relative to the total power were used as HRV indices
174 in the present study. The LFn index represented sympathetic influence while the HFn index
175 represented parasympathetic function in the cardiac activity.
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178 **Statistical analysis**
179    The socio-demographic data were compared between two groups using chi-square test for
180 proportion difference and independent *t*-tests for the difference between groups.
181    The HRV indices (LFn and HFn) were first tested for their normal distribution before
182 conducting a parametric test. The data by groups did not show statistical significance by
183 Kolmogorov-Smirnov test indicating that data exhibited normal distribution. The present data
184 were unequal sample sizes assuming unequal variances. A Welch's *t*-test for unequal variances
185 (Delacre, Lakens & Leys, 2017) was conducted to compare means of HRV indices between the
186 control and LKC groups. The effect size from our preliminary was tested to evaluate the
187 magnitude difference between groups according to Cohen's guidelines (Cohen J., 1988), where d
188 = |0.2| is considered as small, d = |0.5| as medium, and d = |0.8| as large effect size. A power
189 analysis was also conducted based on the effect size. The Welch's *t*-test was carried out using R
190 studio (version 4.1.2).
191    To test whether HRV indices were associated with kratom use variables (duration and the
192 quantity of kratom leaves use per day), Spearman's rank correlation coefficient was performed as
193 some kratom variables showed a non-normal distribution. Since internal factor such as age was
194 relevant to the kratom use variables and HRV indices (Abhishekh et al., 2013), it was included in
195 the correlation test to determine the relationships among of them. Spearman's rank correlation
196 was tested and plotted by Python SciPy library.
197    All statistical significance levels were considered at $p < 0.05$.
198
199

Results

201

Characteristics of the participants and kratom use history
203    The socio-demographic characteristics between control and LKC are shown (**Table 1**).
204 There was no significant difference in age, BMI, smoking, and alcohol drinking history between
205 control and LKC. This means that the backgrounds did not differ between groups, indicating that
206 participants' backgrounds were not significantly different between groups. The average kratom
207 chewing duration of LKC was 18.13 ± 11.04 years (mean ± SD) with the average amount of
208 consumption at 11.65 ± 10.12 leaves/day (mean ± SD).
209
210

211 **Ultra-short HRV in frequency domains between groups**
212    The ultra-short HRV in spectral distributions across frequency components between the
213 representative subjects in control and LKC groups was illustrated **(Figure 1)**. Compared to
214 control levels, LKC appeared to have a lower LFn distribution, whereas an HFn distribution was
215 higher. In quantitative analysis, the Welch's *t*-test was conducted to compare the mean of the
216 indices between groups, as shown **(Figure 2)**. When compared to control levels, LFn in LKC

217   was significantly decreased [$t = 4.5842$, $df = 43.819$, $p < 0.001$, $d = 1.21$ (large effect), observed
218   power = 0.98], whereas HFn was significantly increased [$t = -4.5193$, $df = 45.619$, $p < 0.001$, $d = $
219   1.21 (large effect), observed power = 0.98]. The results suggested that the long-term kratom
220   chewer group exhibited the change in cardiac autonomic function with parasympathetic
221   predominant observed by the increase in HFn and decrease in LFn.
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224
225   **The correlations between changes in HRV indices in LKU and the variables of kratom use**
226   The significant differences in LFn and HFn indices in LKC compared to the controls
227   were further investigated in association with the relevant factors, such as duration, the quantity of
228   kratom leaves use, and age. The duration, except for the quantity of kratom use tended to
229   correlate to LFn positively and HFn negatively. The tendency of the correlation may be a result
230   of age. Since age was likely to correlate with the HRV indices as well. Moreover, age was highly
231   associated with the duration rather than the quantity of kratom use. This could explain why only
232   the duration of kratom use was associated with a higher tendency with those HRV indices.
233   However, all HRV indices were not significantly correlated with these factors tested by
234   spearman's correlation **(Figure 3)**. The results suggested that changes in the HRV indices in
235   LKC were independent of kratom use variables.
236
237

238   # Discussion
239   This preliminary study first evaluated cardiac autonomic function using HRV analysis in
240   the LKC from Southern Thailand compared to that of kratom non-users. Recently, the evidence
241   of autonomic heart regulation in the LKCs remained unexplored. Investigation of HRV was
242   important for a more comprehensive understanding of long-term kratom chewing on the
243   dynamics of cardiac activity. The current findings supported some of the hypotheses that LKC
244   showed some changes in cardiac autonomic function with increased HRV by enhancing HFn and
245   reducing LFn during the resting state compared to control levels. However, the alterations in the
246   HRV indices were not associated with the duration and quantity of kratom use.
247   The present results revealed that long-term kratom chewing increased HRV (increase in
248   HFn and decrease in LFn). A previous study reported that the reduction of sympathetic tone had
249   a similar effect to the increase in parasympathetic reactivity (Levin et al., 2019). The present data
250   demonstrated that LKC changed in cardiac autonomic function that produced parasympathetic
251   predominance. However, the results were inconsistent with the report from regular kratom users
252   who daily consumed brewed kratom tea that produced sinus tachycardia, indicating increased
253   sympathetic activity (Leong Abdullah et al., 2020). This could be a result of different forms of
254   Kratom consumption. The boiling preparation contained diluted kratom content and lower
255   bioactivity in comparison to chewing (Eaimchaloay et al.,2019).
256   Considering the form of kratom chewing, the increased HRV representing the
257   predominant parasympathetic activity in the present study may be supported by the findings of
258   physiological measurements such as EEG activity. Previously, the heart-brain interaction did
259   exist (Jung, Jang & Lee, 2019). Basically, both sympathetic and parasympathetic activities of the
260   autonomic nervous system are regulated by cortical-subcortical pathways (Thayer et al., 2009).
261   A previous study revealed that long-term kratom chewers promoted an increased theta/alpha
262   ratio (increase in theta and decrease in alpha band) at resting-state (Saengmolee et al., 2022). In

263 addition, the change in EEG oscillation was a common response at rest found in opioid users
264 (Phillips, Heming & London, 1994; Wang, Kydd & Russell, 2016). It is well known that opioids
265 enhance parasympathetic activity (Jiang et al., 2009). Based on the evidence, it is possible to
266 confirm that LKC exerted autonomic cardiac modulation by producing parasympathetic
267 predominance.
268   Although the long-term kratom chewers showed the dominant vagal tone observed by
269 enhanced HRV indices (LFn and HFn) were not significantly related to the duration and the
270 amount of kratom leaves used. The results did not support the hypothesis that long-term kratom
271 chewing would have a negative and positive correlation with LFn and HFn, respectively. The
272 present data were not consistent with those of the previous study which showed that EEG
273 activity was strongly associated with the amount of kratom leaves used (Saengmolee et al.,
274 2022). The changes of HRV indices in LKC were not correlated with the amount of kratom use
275 variables but found to be associated with cardiac autonomic modulation. It is likely that the
276 cardiac autonomic function in LKC was modified in adaptation to the sympatho-vagal balance.
277 In particular, the volunteers consumed kratom leaves in daily life for almost 2 decades.
278 Previously, the cardiac autonomic function was shifted from vagal to sympathetic dominance
279 through the heart regulation of peripheral receptors such as arterial baroreceptors (Parati, 2005).
280
281   The present study provided scientific evidence that had never been examined with the
282 practical method for evaluation of cardiac autonomic function with rapidly quantitative
283 measurements in long-term kratom chewers. In the light of the results, long-term kratom chewers
284 were found to show the enhanced parasympathetic activity represented by increased HRV
285 compared to control level. Basically, the higher HRV was considered as a healthy index
286 normally observed in healthy individuals, athletes, and performing yoga (Marian E Papp et al.,
287 2013). In further studies, it would be important to apply the HRV assessment for understanding
288 how alterations in autonomic function correspond with other health outcomes such as emotional
289 self-report and well-being and sleep quality in LKC.
290
291   A limitation might also be discussed in this study. The HRV was analyzed using raw
292 EKG signals recorded in a relatively short time period. Basically, traditional short-term HRV
293 recording recommended a minimum of 5 minutes (Min et al., 2008). However, the ultra-short-
294 term HRV recording (less than 5 minutes) was practical and effective in analysis in the previous
295 study (Lee et al., 2020). Moreover, female kratom users were also surveyed. However, no female
296 respondent was recruited according to the culture and local attitude. They were reluctant to
297 participate. Mainly, female kratom users chew kratom occasionally for traditional remedy
298 (Assanangkornchai et al., 2007; Singh, Narayanan & Vicknasingam, 2016). The plasma
299 concentration of mitragynine that would indicate more accurate amount of consumption was not
300 measured in the present study. However, the averaged amount expressed in leaves per day was
301 also useful for estimation. Further research should be conducted on HRV assessment along with
302 plasma concentration measurement of mitragynine for a more comprehensive understanding of
303 changes in cardiac autonomic function in LKC.
304
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306 **Conclusions**

307   Our preliminary study demonstrated a change in cardiac autonomic function using LFn
308 and HFn parameters to evaluate HRV in LKC. Moreover, the HRV indices in correlation with
309 the duration and quantity of kratom leaves used were also examined. We found that LKC
310 modulated cardiac autonomic function at rest by enhanced parasympathetic activity with a higher
311 HFn, together with a lower LFn. However, the change in cardiac autonomic function was not
312 correlated with the duration and amount of kratom leave consumption indicating that the
313 autonomic regulation may be modified through the adaptation of sympathovagal balance. In
314 further studies, The HFn and LFn parameters might be monitored as particular HRV indices of
315 cardiac autonomic function to evaluate psychological outcomes of treatment in people with daily
316 stress or anxiety. Moreover, these HRV indices are recommended to be used along with other
317 physiological biosignals for additional understanding of correlation between measurable
318 parameters of body response and emotional status.

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Figure 1

The effect of long-term kratom chewing on the HRV in frequency domain analysis.

The distribution of power spectrum in normalized values across the low-frequency (LF; 0.04-0.15 Hz) and high-frequency (HF; 0.15-0.4 Hz) for the representative subjects of controls and LKC
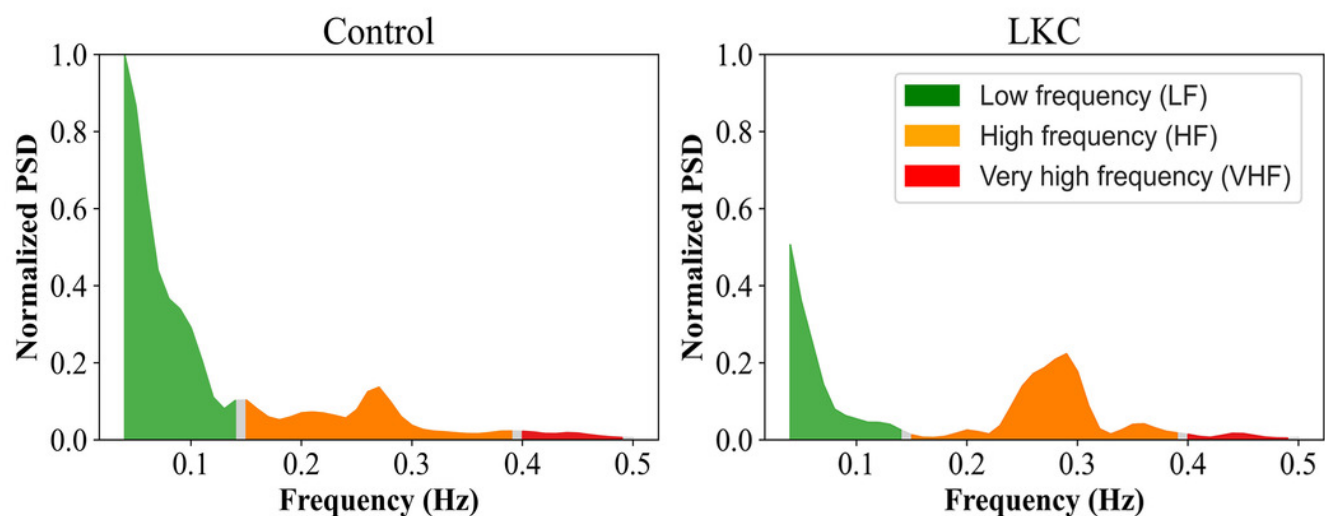
Figure 2

The statistical comparisons between groups' differences for HRV in frequency domain analysis.

The comparisons between groups' differences for the LFn and HFn were tested by Welch's t-test (b). *** denotes $p < 0.001$.
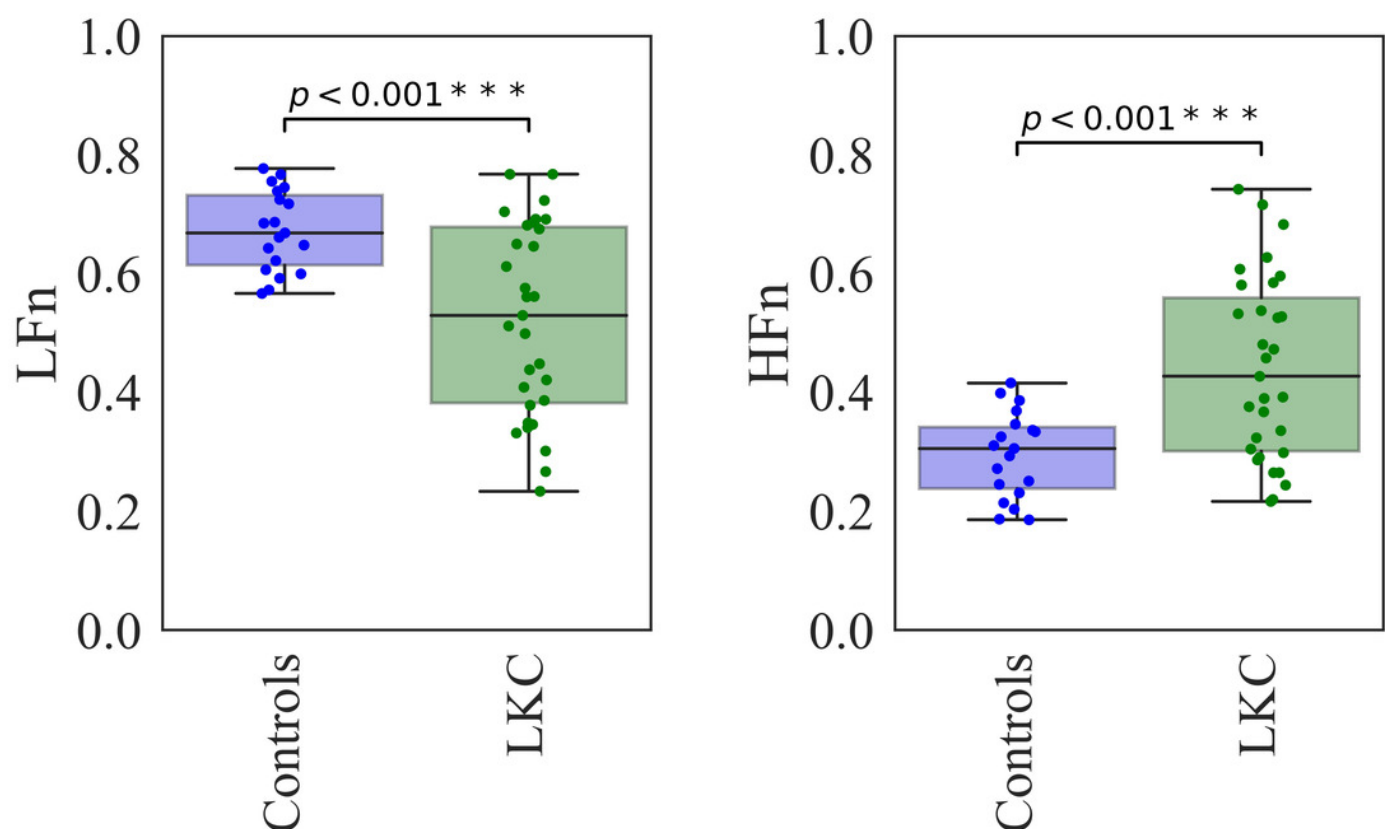
Table 1

The characteristics of participants

Demographic characteristic	Control group	LKC group	*p*-value
Number of subjects, n (%)	19 (38)	31 (62)	-
Sex			
male	19 (100)	31 (100)	
female	-	-	
Age in years, mean ± SD	48.84 ± 10.66	51.32 ± 10.66	0.382 [a]
Body-mass index (BMI) in kg/m2, mean ± SD	23.87 ± 2.82	23.03 ± 4.06	0.430 [a]
Smoking history			0.118[b]
Non-smoking, n (%)	9 (47.30)	8 (25.80)	
Smoking, n (%)	10 (52.70)	23 (74.20)	
Alcohol drinking			0.640 [b]
Non-alcohol drinking, n (%)	8 (42.10)	11 (35.48)	
Alcohol drinking, n (%)	11 (57.90)	20 (64.52)	
Duration of kratom use, mean ± SD	-	18.13 ± 11.04	-
The amount of kratom consumption (leaves/day), mean ± SD	-	11.65 ± 10.12	-

Note: a. independent *t*-test, b. chi-square test

Figure 3

The correlation between HRV indices in LKCs (n =31) and the variables of kratom use, including age.

The pairwise scatter plots are represented in the left corner, while Spearman's correlation coefficients are illustrated in the right corner with the different colors and sizes of the circle shapes.