Bioactive polyphenols in kinkéléiba tea (Combretum micranthum) and their glucose-lowering activities

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Abstract

Herbal tea kinkéléiba prepared from the leaves of Combretum micranthum has been widely consumed in West African countries for its flavor, nutritional and medicinal properties. Under bio-guided screening, the kinkéléiba leaves were chemically investigated using various chromatographic and spectrometric methods that led to the identification of thirteen different flavonoid compounds. Further biological tests illustrated that the identified compounds may have synergistic effects to decrease the expression of phosphoenolpyruvate carboxykinase (PEPCK) mRNA and glucose production in an H4IIE hepatoma cell line, indicating its potential use for insulin-resistant diabetes treatment. Further in vivo study on C57BL/6j mice indicates that kinkéléiba can lower plasma glucose levels in a dose-dependent manner without significant weight loss and toxicity. The ethyl acetate extract in rich of flavonoids could also increase the glucose tolerance (GT) after seven weeks’ administrations. Both in vitro and in vivo experiments support a potential new application of kinkéléiba leaves as an anti-diabetes agent.

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1. Introduction

Combretum micranthum (kinkéléiba) is an undomesticated shrub species found in the Tiger bush region of western Africa. It is a bushy shrub or creeper that can reach up to 20 m in height, and common on cultivated and fallow ground, dominant in sub-Saharan Africa, with higher concentrations in Senegal, Mali, and Burkina Faso [1]. The leaves are harvested from wild growing populations and used as popular traditional herbal
te in several tropical West African savannah countries. Kinkeliba consumed as bush tea, has a pleasant flavor, light to dark green-brown color [2]. The tea is also used in traditional medicine as a general panacea and for diuretic and digestion purposes including gastrointestinal problems, colic, and vomiting [1,3–5]. Previous phytochemical studies indicated that the leaf extracts contain flavonoids including vitexin, iso-vitexin, orientin, homoorientin, myricetin-3-O-glucoside, and myricetin-3-O-rutinoside; alkaloids including stachydrine, hydroxyl-stachydrine, and choline; and sugar alcohols including m-inositol and sorbitol; and flavan alkaloids including kinkeloids A, B, C and D [5–8].

In this investigation, we originally set out to isolate and identify the polyphenols in kinkeliba that we hypothesized may be responsible for its traditional activity through solvent participation and various chromatographic and spectrometric methods including normal pressure chromatography, HPLC/UV/MS and nuclear-magnetic resonance (NMR). Yet, as we also had available a robust series of assays to screen for anti-diabetes activity, we subjected the separated compounds and extracts/fractions to a variety of these assays. The in vitro assays chosen for the bioactivity test of kinkeliba regarding glucose-lowering activity are measuring the percent decrease of glucose production and the down-regulation of PEPCK mRNA in rat hepatoma cells (H4IE) [9]. A diet-induced obesity (DIO) C57BL/6J mouse model was used to further understand the anti-hyperglycemic effect of kinkeliba in vivo. It is a particularly good model of the human metabolic disorder because it develops a syndrome of obesity, accompanied by hyperinsulinemia and hyperglycemia (indicators of Type 2 diabetes), when allowed unfettered access to a high-fat diet [10]. The development of insulin resistance, hyperglycemia, and obesity parallel the progression of Type II diabetes in humans. We hypothesized that kinkeliba’s purported health-promoting properties may be in part due to the presence of polyphenols, and whereas some polyphenols have been shown in other plant species to exhibit anti-diabetic activity, we postulated that leaves of this plant may then also show some positive activity in the treatment of diabetes.

Diabetes mellitus is a chronic disease that affects 29.1 million people or 9.3% of total population in US in 2014 [11]. Specifically, Type 2 diabetes, compromising approximately 90%–95% of all diagnosed cases, often develops from excess body weight and physical inactivity resulting in the body’s ineffective use of insulin, which creates hyperglycemia. There are many factors, including environmental and genetic components, contribute to the hyperglycemia of diabetes [12]. Even if the reasons are not fully understood yet, a prominent defect is that the insulin could not efficiently inhibit hepatic glucose production (HGP) [13]. Increased HGP may be due to increased gluconeogenesis, which involves a series of biochemical reactions catalyzed by several enzymes under the tight regulation of insulin [14]. The first, and also the rate-limiting step of hepatic gluconeogenesis, is controlled by enzyme phosphoenolpyruvate carboxykinase (PEPCK), which converts compound oxaloacetate to phosphoenolpyruvate. Excreted insulin is supposed to strongly repress the expression of PEPCK mRNA through the activation of enzyme phosphoinositide-3 kinase (P3K) pathway, resulting decreased de novo glucose synthesis [15]. However, in diabetes patients, insulin does not effectively inhibit the expression of PEPCK, therefore, resulting in increased blood glucose or hyperglycemia. Phytochemicals or plant extract that can suppress the expression of PEPCK mRNA, may overcome insulin resistance, and lower the glucose production from gluconeogenesis [9]. Therefore, the focus of this research shifted to determine whether bioactive polyphenols in kinkeliba exhibit anti-diabetic activity in vitro and in vivo.

2. Materials and methods

2.1. Chemicals and biochemicals

8-(4-Chlorophenylthio)-cAMP, dexamethasone, sodium pyruvate, sodium lactate, and ABTS (2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) were purchased from Sigma–Aldrich (St. Louis, MO). Human insulin was purchased from Eli Lilly (Indianapolis, IN). Reagents used in RT-PCR, including the enzymes, were purchased from Stratagene (La Jolla, CA). The H4IIE cell line (ATCC CRL-1548) was provided by the American Type Culture Collection (Manassas, VA).

2.2. Plant material

Kinkeliba samples utilized for this study was collected from shrubs within a localized population in Leen, Pout, in central Senegal. Plant materials were gathered by the NGO ASNAPP-Senegal with members of the community and with prior written consent from the community and community leaders. Locally, communities often dry the leaves and branch together forming a long bundle and then sold locally as an herbal tea. The leaves and branches were manually harvested, air-dried under shade in ambient conditions and off the ground on white tarp. Once dried, the leaves were manually separated from the branches, and cleaned. The trees from which the leaves were harvested were taxonomically identified as C. micranthum by the authors who have been working with this same plant population and species in Senegal with the community as an indigenous herbal tea to provide additional local income opportunities. The dried leaf material were originally air-shipped to South Africa where the leaves were processed by Cape Natural Teas, Cape Town, South Africa and packaged into a Mpuntu line of African herbal teas and then air-shipped to Rutgers University for analysis. Additional dried leaves from same plant populations were air-shipped directly from Dakar, Senegal to Rutgers University for analysis.

2.3. General methods

1D and 2D NMR spectroscopy was performed on a Bruker Avance 400 MHz spectrometer (Billericia, MA). All organic solvents used for chromatographic separation are HPLC grade (Fisher Scientific, Springfield, NJ). Column chromatography was performed using silica gel (230–400 mesh; Selecto Scientific, Suwanee, GA), Sephadex LH-20 (25–100 μm, Sigma-Aldich, St. Louis, MO) and Polyamide CC 6 (50–160 μm, Sorrent Technologies, Atlanta, GA). HPLC grade water was prepared using a Millipore Milli-Q purification system (Millipore Corp, Bedford, MA) and used for all experimental procedures.
Preparative HPLC was performed on a Waters 600/2487 system with a UV detector and Microsorb C18 column (Varian, 10 µm, 41.4 x 300 mm). Analytical HPLC was performed on Waters 2695/2996 (Milford, MA) with diode array detector (DAD). LC-MS detection was performed on an Agilent 1100 series (Waldbronn, Germany) with DAD detector, MSD trap with an electrospray ion (ESI) source under positive mode. Analytical column is Microsorb C18 column (Varian, 5 µm, 4.6 x 250 mm).

2.4. Extraction and isolation

The dried leaves (1.0 kg) were ground to fine powder and extracted with ethanol two times and a third time with an 80% ethanol (v/v) solution by maceration for 24 h at room temperature. The filtrates were combined and concentrated under reduced pressure. The crude extract was dissolved in water/ethanol (95:5, v/v) and partitioned between hexane (labeled as LH), chloroform (LC), ethyl acetate (LE), and n-butanol (LB), with the remaining water fraction as LW. Antioxidant activity of the fractions with potent antioxidant activity may also present subject to silica gel chromatographic column (5%)/C2 to give compound 10. LE-10 (4.46 g) was subjected to silica gel chromatographic column (5%)/C2 to give residue (14.40 g), which was applied to polyamide column and eluted with a gradient of 100% acetonitrile/water with 0.1% formic acid) to give 12 fractions, labeled as LE-1-10. The mixture of 3', 4', 5', 7- pentahydroxyflavon (3): UV \( \lambda_{max} \) 275 nm. ESI-MS: m/z 291 [M+H]+, 139 (RDA fragment, 1,3A); 1H NMR (400 MHz, MeOD) and 

\[ \text{Supplementary Material S6; } \]

2'-O-galloylisorvitexin (10): UV \( \lambda_{max} \) 216, 269, 338 nm. ESI-MS: m/z 585 [M+H]+, 313, 415, 433, 465. 1H NMR (400 MHz, MeOD) δ 6.41–6.60 (1H, s, H-3), 6.07–6.22 (1H, s, H-6), 7.81, 8.06 (2H, d, J = 8.7 Hz, H-2, 6), 6.96 (2H, d, J = 8.7 Hz, H-3, 5), 5.20, 5.32 (1H, d, J = 10 Hz, H-1), 5.62, 5.71 (1H, t, J = 10 Hz, H-2), 3.84 (1H, t, J = 9.2 Hz, H-3), 3.76 (1H, t, J = 9.2 Hz, H-4), 3.56 (1H, m, H-5), 3.85, 4.02 (2H, H-6), 6.89, 6.69 (2H, s, H-2'), 3.13 NMR (100 MHz, MeOD) δ 166.75 (C-2), 103.76 (C-3), 184.20 (C-4), 162.79 (C-5), 99.19 (C-6), 164.23 (C-7), 103.76 (C-8), 158.57 (C-9), 105.69 (C-10), 123.83 (C-1'), 130.19 (C-2'), 117.05 (C-3'), 162.79 (C-4'), 117.05 (C-5'), 130.19 (C-6'), 72.56 (C-1'), 74.19 (C-2'), 78.09 (C-3'), 73.38 (C-4'), 83.25 (C-5'), 63.14 (C-6'), 121.42 (C-1'), 110.34 (C-2'), 146.27 (C-3'), 139.70 (C-4'), 146.27 (C-5'), 110.34 (C-6'), 166.75 (C-7'); 2'-O-galloylisorvitexin (11): UV \( \lambda_{max} \) 216, 269, 338 nm. ESI-MS: m/z 585 [M+H]+, 313, 415, 433, 465; 1H NMR (400 MHz, MeOD) δ 6.48 (1H, s, H-3), 6.39 (1H, s, H-8), 7.74 (2H, d, J = 8.7 Hz, H-2, 6), 6.87 (2H, d, J = 8.7 Hz, H-3, 5), 5.16 (1H, d, J = 10 Hz, H-1), 5.77 (1H, H-2'), 3.76 (1H, t, J = 9.3 Hz, H-3), 3.62 (1H, t, J = 9.3 Hz, H-4), 3.51 (1H, m, H-5), 3.80, 3.94 (2H, H-6), 6.94 (2H, s, H-2', 6'); 13C NMR (100 MHz, MeOD) δ 166.18 (C-2), 103.92 (C-3), 183.87 (C-4), 162.70 (C-5), 108.02 (C-6), 164.50 (C-7), 158.81 (C-8), 104.97 (C-9), 123.18 (C-1'), 129.46 (C-2'), 116.99 (C-3'), 162.70 (C-4'), 116.99 (C-5'), 129.46 (C-6'), 73.87 (C-1'), 73.98 (C-2'), 78.18 (C-3'), 71.87 (C-4'), 82.88 (C-5'), 62.86 (C-6'), 121.51 (C-1'), 110.34 (C-2'), 146.23 (C-3'), 139.64 (C-4'), 146.23 (C-5'), 110.34 (C-6'), 167.61 (C-7'); 2'-O-galloylisorvetin (12): UV \( \lambda_{max} \) 216, 269, 338 nm. ESI-MS: m/z 601 [M+H]+, 329, 431, 449, 481.
2”-O-galloylhomoorientin (13): UV λ_max; 216, 269, 338 nm. ESI-MS: m/z 601 [M + H]⁺, 329, 431, 449, 481

2.6. Cell culture

The H4IIE hepatoma cells (ATCC CRL-1600) were routinely cultured in Dulbecco’s modified Eagle’s medium supplemented with 2.5% (v/v) newborn calf serum, 2.5% (v/v) fetal calf serum and grown at 37 °C in humidified 5% CO₂ atmosphere.

2.7. RNA extraction and quantitative real-time polymerase chain reaction (qPCR)

The H4IIE cells were plated in 24-well tissue culture plates (Greiner Bio One, Monroe, NC) and were grown to near confluence. A treatment of 500 mM dexamethasone and 0.1 mM 8-CTP-cAMP (Dex-cAMP) for 8 h was used to induce PEPCK gene expression. The cells were co-treated with plant extract (100 µg/mL), subfractions (100 µg/mL), isolated compounds (20 µg/mL) or insulin (10 nM).

Total RNA was extracted from treated H4IIE cells using Trizol reagent (Invitrogen), treated with DNase I (Invitrogen) to remove any traces of DNA contamination, and quantitated spectrophotometrically using the NanoDrop system (NanoDrop Technologies). Gel electrophoresis was used to assess the quality of RNA sample. 2.5 µg RNA from each sample was used as template to synthesize cDNA using StrataScript reverse transcriptase (Stratagene, La Jolla, CA), following the manufacturer’s protocol [9].

PCR reaction was comprised of 5 µL of each 4-fold diluted cDNA sample, 0.5 µL of 6 µM primers (IDT, Coralville, IA), and 12.5 µL of Brilliant SYBR Green PCR master mix (2X; Stratagene) supplemented with green jump-start Taq polymerase. ROX (Stratagene) was used as a reference dye. qPCR were performed on MX3000p system (Stratagene) with the following steps: initialisation (1 cycle at 50 °C for 2 min, 1 cycle of 95 °C for 10 min), amplification (40 cycles of 15 s at 95 °C and 1 min at 60 °C), and dissociation (1 cycle at 95 °C for 1 min, 55 °C for 30 s, and 95 °C for 30 s). Non-template control and non-RT control were performed in each experiment to rule out the potential contamination. The primers were designed using the Primer Express software (Applied Systems, v. 2.0, Foster City, CA) as follows: β-actin: 5’-GGG AAA TGG TGC GTG ACA TT-3’ (sense) and 5’-GGC GCA GTG GGC ATC TC-3’ (anti-sense); PEPCK: 5’-GCA GAG CAT AAG GCC AAG GT-3’ (sense) and 5’-TTG CGG AAG TTG TAG CCA AA-3’ (anti-sense). The relative mRNA expression of PEPCK were analyzed using the DDC₇ method with β-actin as a housekeeping gene as previously described [9]. The PEPCK gene expression of the Dex-cAMP treatment (positive control) was defined as 1, any expression level less than 1 from the treatment indicated the inhibition of the mRNA expression of PEPCK.

2.8. Glucose production assay

The glucose production assay was performed as previously published with some modifications [9]. Briefly, H4IIE cells treated with Dex-cAMP were added 10 nM insulin, plant extracts, sub-fractions or purified compounds for 5 h at 37 °C in 5% CO₂. The medium was removed, and the cells were rinsed with phosphate-buffered saline. Glucose production buffer (glucose-free Dulbecco’s modified essential medium, pH 7.4, containing 20 mM sodium lactate and 2 mM sodium pyruvate without phenol red) supplemented with dexamethasone and 0.1 mM 8-CTP-cAMP in the presence of 10 nM insulin (Sigma), and plant extracts, sub-fractions, or purified compounds were added, and cells were incubated for an additional 3 h at 37 °C in 5% CO₂. At the end of the incubation, the glucose concentration was measured from 0.5 mL of culture medium using the Amplex Red glucose assay kit (Invitrogen). The protein concentration quantitated by the Bradford method was used for correction of cell number.

2.9. Animal experiments

All animal experiments were performed according to procedures approved by the Rutgers Institutional Animal Care and Use Committee (protocol #04-023). Ten-week-old male C57BL/6j mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and maintained on a high-fat diet containing 60% fat-derived calories (D12492, Research Diets, New Brunswick, NJ) with 12-hour light and dark cycles. The animals were randomized into 3 groups of 10 animals each. The control group was oral gavaged daily with the vehicle solution alone (5% DMSO) and two treatment groups were oral gavaged with 200 mg/kg of the appropriate treatment: crude extract (L) and EtOAc fraction (LE). The animals were weighed weekly for the duration of the experiment. Plasma glucose levels were measured at week 0 and 4 in sub-mandibular vein blood samples using a glucometer (Accu-Chek Advantage, Roche Diagnostics, Indianapolis, IN). A 6 h fast was necessary to allow blood glucose concentrations to arrive at the basal level, plasma glucose concentrations were measured immediately before as 0 h and at 3, 6 h following the treatments of metformin, L, and LE. The oral glucose tolerance test (OGTT) was performed at week 7. The mice were fasted overnight (around 16 h) followed by oral gavage with 2.0 g/kg glucose solution. Glucose levels were subsequently measured using a glucometer at 0, 40, 80, and 130 min after glucose administration.

2.10. Data presentation and statistical analysis

Statistical analyses of the experimental observations, expressed as means ± SEM, can be assumed to be one-way ANOVA followed with a Tukey’s multiple means comparison test, unless otherwise indicated. Treatments were considered significantly different if P < 0.05.

3. Results and discussion

3.1. Structure elucidation

Polyphenol compounds exhibit a wide range of pharmacological activities such as anti-cancer, anti-inflammatory, and anti-diabetes [22–25]. In our study, ABTS scavange assay was applied for in vitro bio-guided screening based on previous research [26]. Ethyl acetate (LE) fraction was found to exhibit superior activity compared to others (Supplementary Material
Subsequent chemistry analysis indicated that different polyphenol compounds exist in the ethyl acetate fraction (LE) of kinkeliba leaves (Fig. 1). The structure of each compound was elucidated by various spectrometric methods including UV, MS and NMR spectroscopy, or compared with commercial available standards on HPLC. Four flavan compounds were identified as (-)-epicatechin, (-)-epigallocatechin, 3', 4', 5', 7-pentahydroxyflavan and 3', 4', 5', 7-tetrahydroxyflavan using NMR, and the results correlate very well with previous literature reports (Supplementary Material S6) [27,28]. They share identical fragment ions \(^{139}\) (m/z = 139) on mass spectra, which further confirms their flavan identities (Fig. 2A). The chiral centers of compounds epicatechin, epigallocatechin were unambiguously elucidated through the comparison with authentic standards on HPLC (Supplemental Material). This is the first report that these flavan compounds are naturally occurring in kinkeliba. Except the flavans, a number of flavone C-glycosides were separated, characterized and identified. Compound 5, vitexin was unambiguously identified based on UV and MS data and further comparison with authentic standard on HPLC. Compound 6, 7, 8 were tentatively identified as isovitexin, orientin, homoorientin based on their UV and MS spectra along with literature reports. They have identical UV absorption peaks at ~216, 269, 338 nm and characteristic fragment ions \(^{313}\text{+}^{329}\) (m/z = 313 or 329) and \(^{359}\text{+}^{375}\) (m/z = 359) ions of C-glycosylflavones (Fig. 2B) [29,30]. The O-galloyl derivatives (10–11) of C-glycosylflavones were also separated on HPLC and identified using MS. The structures of 10 and 11 were further confirmed using NMR. The galloyl derivatives of C-glycosylflavones also share identical UV absorption peaks at ~216, 269, 338 nm [30]. The mass fragment ion \(^{517}\text{+}^{533}\) (m/z = 481 or 465) formed inside the glucose ring and \([M\text{-galloyl}+H]\text{+}^{517}\) ions (m/z = 433 or 449) further confirms that galloyl group is attached at the 2' position (Fig. 2C) [31]. Fragment ion (m/z = 313, 329) from both \(^{2}X\text{+}\) cleavage and galloyl group removal was also observed on the spectra. These O-galloyl derivatives are revealed in kinkeliba for the first time. One known flavonol compound myricetin-3-O-glucoside (9) was also purified and confirmed using NMR [32]. The mass spectra of C-glycosylflavones and their O-galloyl derivatives are illustrated in S4 (Supplementary Material).

3.2. Inhibition of PEPCK mRNA expression

The ethanol crude extract (L), ethyl acetate fraction (LE), and its sub-fractions were tested for inhibitory activity of Dex-cAMP induced PEPCK mRNA expression on H4IIE hepatoma cells line. The synthetic compound glucocorticoid dexamethasone and cAMP analog 8-CTP-cAMP were used to up-regulate the expression of PEPCK gene. After the treatment of insulin or tested compounds on H4IIE cell line, the decreased expression levels of PEPCK indicated their inhibitory activity of gluconeogenesis and potential use for diabetes treatment. Cells without any treatments were used as control group and to indicate the basal level of PEPCK expression. \(\beta\)-Actin was used as an internal standard in this assay since its expression won't be affected after the treatments. The crude extract would slightly inhibit the expression of PEPCK at low concentration, and around 16% inhibition was observed at the concentration of 400 \(\mu\)g/ml (Fig. 3A). The ethyl acetate fraction

![Fig. 1 – The flavonoids isolated from ethyl acetate fraction (LE) of kinkeliba leaf extract.](image-url)
further increased the inhibitory activity and dose dependent manner was observed at concentration above 75 μg/ml. The inhibition of LE at 125 μg/ml was up to around 60% (Fig. 3A).

To further identify the main components responsible for the suppression of the mRNA expression of PEPCK, the ethyl acetate extract was further separated into different fractions. Their inhibitory effect and the main compounds contained in each fraction are illustrated in Fig. 3B. The bioactivity of different sub-fractions varies and the ones containing compounds 1–3 and 10–13 showed the highest inhibitory activity (around 83% and 76% inhibition, respectively). The fraction containing compounds 1–3 (100 μg/ml) showed stronger inhibitory effect than insulin (10 nM). The major components contained in sub-fraction 1–3 are (-)-epigallocatechin, (-)-epicatechin, and 3’, 4’, 5’, 5, 7-pentahydroxyflavan. Further isolation of each single component for bioassay indicated that compound (-)-epicatechin (2) possessed the best inhibitory activity compared to compound 1, 3, 4, with 32% inhibition at 20 μM. The mixture of different flavones (100 μg/ml) including 2”-O-galloylvitexin (10), 2”-O-galloylisovitexin (11), 2”-O-galloyorientin (12), 2”-O-galloylhomoorientin (13) also showed potent inhibition activity, even better than insulin of 10 nM.
Sub-fractions 10–11 and 12–13 have relatively lower inhibitory activities compared to 10–13, which is a combination of 10–11 and 12–13. These results suggest that sub-fractions 10–11 and 12–13 may present the inhibitory activity through two or more different mechanistic pathways. While each sub-fraction did not strongly inhibit the expression of PEPCK mRNA, when combined their inhibitory activity increased significantly, suggesting that these compounds may have synergistic effects on the inhibition of PEPCK mRNA expression through two or more different mechanistic pathways.

3.3. Inhibition of glucose production

To further assess the effect of down-regulation of PEPCK gene expression on glucose production in vitro, compounds pyruvate and lactate, which could be used by H4IIE hepatoma cells for gluconeogenesis, were added to the cell medium. Phytochemicals in the extract, which inhibited the mRNA expression of PEPCK, when combined their inhibitory activity increased significantly, suggesting that these compounds may have synergistic effects on the inhibition of PEPCK mRNA expression through two or more different mechanistic pathways.

3.4. Animal experiments

3.4.1. Body weight and tissue weight

The daily treatments of kinkeliba samples (L, LE) did not show a significant effect on the body weight change of the animals over the six weeks. The treatment groups have similar weight gain with the control group at all time points we measured, though only week 0 and week 6 are included (Supplementary Material). At week 6, mice fed with LE extract has slight lower body weight and tissue weight compared with the control group indicating kinkeliba could slightly slow down the weight gain, which to some extent correlates with its traditional use for weight loss. This lack of significant weight difference also suggested that the decrease in plasma glucose level is not a result of decreased weight, but rather a therapeutic effect of treatment on glucose production. Similar trend was also observed on the tissue weight, which further supported the non-toxicity of kinkeliba and irrelevance between body/tissue weight and plasma glucose levels.
3.4.2. In vivo plasma glucose levels suppression

The acute blood glucose levels were determined at week 0 and 4. Metformin, an anti-diabetic drug for the treatment of type 2 diabetes, is used as a positive control [33]. As shown in Fig. 4A, after the treatment of leaf extracts, the blood glucose concentration gradually decreased and is comparatively lower than the control group. The ethyl acetate group is slightly more potent than the crude extract based on its higher concentration of polyphenol compounds mentioned above. The blood glucose level decreases by 13.4% at 3 h and 26.1% at 6 h after the treatment of ethyl acetate fraction. Even if the plant extract is not as potent as anti-diabetic drug molecule, metformin, its therapeutic effects on glucose lowering would also be explored in the future and applied as nutraceutical supplements.

3.4.3. Glucose tolerance test

The oral glucose tolerance test was performed after six weeks’ administration of kinkéliba leaf extracts. Administration of 2 g/kg glucose to C57BL/6J mice increased the serum glucose from 159 ± 35 mg/dl to 348 ± 66 mg/dl at 40 min, 356 ± 84 mg/dl at 80 min, and returned back to 243 ± 13 mg/dl at 130 min (Fig. 4B). The administration of crude extract (L, 200 mg/kg) suppressed the elevation of serum glucose levels, with glucose level of 336 ± 50 mg/dl and 315 ± 62 mg/dl at 40 min and 80 min, respectively. As expected, the ethyl acetate extract (LE) with concentrated polyphenol compounds showed stronger inhibition of the glucose level upon glucose intake than crude extract. As shown in Fig. 4B, the blood glucose concentration was 311 ± 69 mg/dl and 297 ± 58 mg/dl at 40 and 80 min, respectively. At 80 min, the glucose level of mice treated with LE was 14.5% lower compared with the control group, another indicator of improved glucose metabolism. The results clearly suggested that the kinkéliba could slow down the increase of glucose levels.

4. Conclusions

Compared with previous works, this study comprehensively investigated the major phytochemicals, especially the polyphenols, contained in African tea plant kinkéliba [34]. Under bio-guided screening, this investigation led to the successful identification of thirteen polyphenols, in which (-)-epigallocatechin, (-)-epicatechin, 3’, 4’, 5’, 7-pentahydroxyflavan, and 3’, 4’, 5’, 7-tetrahydroxyflavan were discovered for the first time in this plant species. Further biological activity studies on purified fractions, which contain different polyphenol compounds, present more in-depth knowledge about the specific functions of polyphenol components. An anti-diabetic activity of kinkéliba was identified and validated in vitro through its inhibition of PEPCK mRNA expression and glucose production in H4IIE rat hepatoma cell line. The ethyl acetate sub-fractions containing compounds 1–3, 10–13 showed impressive inhibitory of PEPCK mRNA expression and underlying synergistic effects may be involved. Compound 2 (-)-epicatechin has the best in vitro PEPCK gene inhibition activity and glucose production inhibition activity. Except PEPCK, other key factors in the development of diabetes, such as Phospho-PKA, phospho-CREB, and PGC-1, should be also further investigated in the future [35–37]. As reflected by the consistence of body weight and tissue weight in vivo experiment, the kinkéliba has no obvious toxicity. Administration of kinkéliba extract, especially the ethyl acetate extract, could decrease the blood glucose levels in vivo. At the end of six weeks’ experiment, the mice administrated with kinkéliba also showed better glucose tolerance compared with the control group. All these results clearly point to noteworthy in vitro and in vivo anti-diabetic activity. This is the first report of glucose-lowering activity by kinkéliba herbal tea (C. micranthum) and should be explored in further detail, especially the underlying therapeutic mechanisms. Based on its demonstrated bioactivities and the high-demand for the prevention or treatment of diabetes, kinkéliba would also be of interest as nutraceutical supplement in the future and as an herbal tea and in other beverage and food products with this data expanding upon its traditional applications.

Conflicts of interest

All contributing authors have no conflicts of interest to declare.
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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jfda.2017.05.009.

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