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"DAK", a traditional decoction in Palau, as adjuvant for patients with insufficient control of diabetes mellitus type II

Christopher Kitalong[a], Renata Campos Nogueira[b], Jeanne Benichou[c], Victor Yano[a], Vanessa Espangel[a], Pearl L. Marumoto[a], Josepha Tiobech[a], Joëlle Houriet[d], Carla Rudigier[e], Bertrand Graz[a,b,*]

[a] Pacific Academic Institute for Research, 822 Ernguul Rd., Koror, Palau
[b] Foundation Antenna Technologies, Avenue de la Grenade 24, 1207 Geneva, Switzerland
[c] ENSAT-National Institute of Agronomy and Environment of Toulouse, Avenue de l'Agrobiopole, BP 32607 Auzeville-Tolosane, France
[d] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, CMU, Rue Michel Servet 1, 1211 Geneva, Switzerland
[e] Institute of Food, Nutrition and Health, ETH Zurich, Schorenstrasse 16, 8603 Schwerzenbach, Switzerland

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ABSTRACT

Ethnopharmacological relevance: Can a medicinal plant be useful when standard modern treatment is insufficient? After a population survey in the Republic of Palau (retrospective treatment-outcome study, following the reverse pharmacology approach) on local treatments and associated outcomes for diabetes, a traditional drink made with *Phaleria nisidai* Kaneh and several other plants called "*Delal A Kar*", (meaning "mother of medicine") appeared as a promising therapy. This is the first clinical study on a standardized version of "Delal A Kar" called *DAK*.

Aims/hypothesis: This is a study of the effect of DAK as adjuvant therapy when diabetes control is insufficient.

Methods: In this randomized, double blind, crossover study conducted in Koror, Palau, DAK or placebo was assigned to 68 patients with type II diabetes treated with oral hypoglycemic agents and with insufficient glycaemic control. All patients received instructions on how to improve their diet and a home glucometer for blood glucose follow-up.

Results: Fifty-five patients completed the study and significant improvements were observed in both groups over the 12 weeks follow-up period: weight decreased an average of 2 to −4.5 pounds ($p < 0{,}001$) and HbA1C also decreased from 9.7% to 7.8% ($p < 0{,}001$), with a consistent trend toward better outcomes after DAK, as compared to placebo. The average effect of Ongael was 0.5% (SD 2.5) decrease of HbA1C. Furthermore, seventy-five percent (41/55) of the patients reduced their HbA1C by at least 0.7% at 12 weeks.

Conclusion: The observed trends in this trial suggest that poorly controlled diabetic patients improved their control of diabetes within 12 weeks when drinking DAK for at least 6 weeks. The drink DAK, in addition to the usual prevention activities of special diet and physical exercise, was followed by improvement of diabetes control (HbA1C) and decrease of blood pressure and weight.

Trial registration: The study protocol was approved by the Institutional Ethical Committee and registered by international Australia and New Zealand trial registry (Request Number: 369395)

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1. Introduction

The Republic of Palau, a relatively isolated island in the Pacific, has a very fast rising rate of non-communicable diseases (particularly obesity, diabetes mellitus type II and hypertension), making it a well-suited place for research on this theme. Local high-level commitment is observed in the country, since the President of Palau declared a state of emergency on non-communicable diseases in 2011. Many public health

Abbreviations: BP, blood pressure; DBP, diastolic blood pressure; DAK, single species standardized version of "Delal A Kar", a Palauan traditional decoction; HDL, high density lipoprotein; LDL, low density lipoprotein; NCD, Non-communicable diseases; NS, non significant; RCT, randomized controlled trial; RTO, Retrospective treatment outcome study; SBP, systolic blood pressure; WHO, world health organization; UHPLC, ultra high performance liquid chromatography; UV-PDA, ultraviolet-photodiode array; ELSD, evaporative light scattering detection; ESI-HRMS-TOF, electrospray ionisation – high resolution mass spectrometry – time of flight

* Corresponding author at: Foundation Antenna Technologies, Avenue de la Grenade 24, 1207, Geneva, Switzerland.
E-mail address: medicine@antenna.ch (B. Graz).

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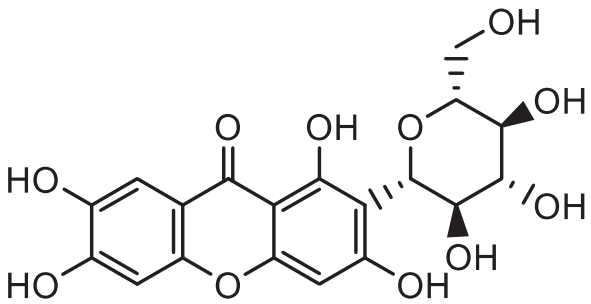

Fig. 1. Structure of the mangiferine (**1**).

interventions at both individual and population levels have been implemented in order to overcome the problem of obesity and life-style-related diseases. These programs have had very limited success and innovative approaches need to be explored (Ichiho et al., 2013).

In a country-wide population study, Delal A Kar, a Palauan traditional drink made with *Phaleria nisidai* and other plants, was recorded as the local treatment with the best reported outcome in case of diabetes in Palau, hence it could be selected for further clinical study (Graz et al., 2015).

Prior phytochemical analysis of *Phaleria nisidai* Kaneh (or *P. nisidae*) has revealed the presence of flavones, benzophenones (Kitalong et al., 2007, 2012), xanthones (Matsuda, 2005; Kitalong et al., 2007, 2012), acylglucosylsterols (Matsuda et al., 2005) fatty acids (Kitalong et al., 2007) and daphnane diterpene esters (Kulakowski et al., 2015). There is a high mangiferin (Fig. 1) content in *P. nisidai* aqueous extract (Kitalong et al., 2012). Mangiferin lowered the blood glucose level in an animal model with type-2 diabetes 3 weeks after oral administration (Miura et al., 2001). From Hou's study (Hou et al., 2012) on mangiferin and sitagliptin in diabetic rats, several interesting properties of mangiferin were observed: improved glucose tolerance test, inhibition of DPPIV enzyme, increased insulin secretion and increased β-cell/islet area ratio. In addition, the following properties of mangiferin were observed in animal studies: alpha amylase inhibitory effect (IC50 value 74.35 ± 1.9 μg/mL) and alpha glucosidase inhibitory effect (IC50 41.88 ± 3.9 μg/mL) when compared with standard drug acarbose (IC50 83.33 ± 1.2 μg/mL) (Dineshkumar et al., 2010).

As shown in our previous RTO study, Palauans commonly use the local recipe of interest and volunteers were recruited and agreed to join the randomized clinical study. This provided an opportunity to measure clinical effects of *P. nisidai* using standard clinical study methods (Graz et al., 2007). One problem in designing such a comparative study of a local medicine well known in a small population is developing a credible placebo. Thus, the study objective was not only to develop a protocol and finding preliminary results but was also to properly test the placebo quality. With these objectives, *DAK* was assessed for the first time as adjuvant therapy for patients with insufficient diabetes control.[1]

2. Materials and methods

2.1. Phaleria nisidai Kaneh leaves collection

The leaves were collected throughout the study duration in Palau. Reference specimens are held at Belau National Museum Herbarium and New York Botanical Garden herbarium. Non-UV dried leaves were used for easier storage and reproducibility.

[1] According to the American Diabetes Association, "lowering A1C to below or around 7% has been shown to reduce microvascular and neuropathic complications of type 1 and type 2 diabetes. Therefore, for microvascular disease prevention, the A1C goal for nonpregnant adults in general is < 7%. (grade A) (Ref.: Standards of Medical Care in Diabetes – 2009. Diabetes Care January 2009 32:S6S12).

2.2. Preparation of DAK

The tested product, DAK, is smallest common denominator of several Delal A Kar recipes: a standardized decoction of *Phaleria nisidai* Kaneh., or *Ongael* in Palauan, leaves prepared following a traditional recipe. 60 g of dry *P. nisidai* leaves for 10 l of water were brewed in an induction boiler for one hour and allowed to cool down to room temperature. Once at room temperature the decoction was filtered and transferred in 500 mL new plastic bottles and sealed. All processes were completed at the PAIR brewing facility, using leaves from the same trees dried in non-UV conditions.

2.3. Mangiferin detection

The standardized decoction was prepared according to the previously mentioned protocol for *DAK* and was then lyophilized.

2.3.1. UHPLC-UV-PDA-ELSD

UHPLC measurements were performed using an Acquity UPLC system (Waters®, Milford, MA, USA), with a binary pumping system, an auto-sampler, a column manager with a pre-column heater, a UV–PDA and an evaporative light scattering detector (ELSD), Sedex 85 (Sedere® LT-ELSD, Alfortville, France). The system was controlled using Empower® 3 Software. UV-PDA detection was performed from 210 to 500 nm (1.2 nm resolution). The temperatures in the auto sampler and in the column oven were fixed at 10 and 40 °C, respectively. The binary system was using two mobile phases: water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B) (ULC/MS grade, Biosolve Chimie SARL, Dieuze, France). A solution containing rutin (Fluka AG, Buchs, Switzerland) and glycyrrhetinic acid (Carl Roth, Karlsruhe, Germany) at 500 μg/mL) standards was injected before the analyses to verify measured retention times and to allow for comparison with other chromatographic devices. Separation was achieved on an Acquity UPLC BEH C_{18} column (1.7 μm, 2.1×150 mm; Waters®, Milford, MA, USA) with a 30 min linear gradient of 95% of A and 5% of B,, followed by a 10 min isocratic step with 95% of B and a 10 min re-equilibration step. Injection volume was set at 2 μL, the flow rate was fixed at 0.46 mL/min. The lyophilized DAK was suspended in water and methanol (70/30 v/v) at 10 mg/mL for ELSD detection and at 1 mg/mL for UV-PDA detection. Spiking experiment with mangiferin and comparison with pure mangiferin were performed with the same condition and are presented in the Supplementary material.

2.3.2. UHPLC-UV-HRMS-TOF analysis

The metabolite profiling of the lyophilized DAK was performed on a Waters® Acquity UPLC system coupled to a Waters® Micromass LCT Premier Time-Of-Flight (TOF) mass spectrometer (Waters®), equipped with an electrospray interface (ESI). The chromatographic conditions were similar to those used for the UHPLC-UV-PDA-ELSD metabolite profiling. Analyses were performed in negative ionisation mode in the 100–1300 Da range with acquisition times of 0.3 s in centroid mode. The ESI conditions were set as followed: capillary voltage 2400 V, cone voltage 40 V, source temperature 120 °C, desolvation temperature 300 °C, cone gas flow 20 L/h, desolvation gas flow 800 L/h and MCP (microchannel plate) detector voltage 2450 V. MassLynx software 4.1, SCN 639 (Waters®, Milford, USA) was used to drive the system. A solution containing both rutin (20 μg/mL) and glycyrrhetinic acid (10 μg/mL) was injected before the analyses to check the reliability of the measured retention time. The lyophilized DAK was resuspended in water and methanol (70/30 v/v) at 1 mg/mL.

2.4. Preparation of DAK and placebo plastic bottles

The placebo drink was prepared with distilled water and food color. McCormick® Egg yellow food color with small additions of red and blue food color drops were used to create the *DAK* color profile in the