Fatty acids composition and in vivo biochemical effects of *Aleurites moluccana* seed (Candlenut) in obese wistar rats

Matheus Camargos de Britto Rosa¹, Paula Reis Ribeiro¹, Viviam de Oliveira Silva², Danubia Aparecida de Carvalho Selvati-Rezende³, Tácio Peres da Silva⁴, Fernanda Rezende Souza¹, Maria das Graças Cardoso³, Josilene Nascimento Seixas², Eric Francelino Andrade²,⁵, Vanessa Pardi⁶, Ramiro Mendonça Murata⁶ and Luciano José Pereira¹,²*

**Abstract**

**Background:** Candlenut (CN) has been used indiscriminately for weight loss. In vivo effects of CN in different doses are scarce.

**Objective:** To evaluate the effects of CN ingestion in obese rats.

**Design:** Thirty animals (obese and non-obese) received one of three different types of treatments: placebo, CN ingestion in a popular therapeutic regimen (8 days with oral administration of 0.2 mg/kg followed by 20 days with doses of 0.4 mg/kg), and ingestion of a doubled popular dose—called 2CN. Treatment was maintained for 28 days.

**Results:** The fatty acid profile of CN indicated mainly linolelaidic and palmitoleic acids. Rats receiving CN and 2CN showed reduced plasmatic levels of glucose and lipoproteins (p < 0.05). A dose-dependent carcass fat reduction was observed (p < 0.05). Blood levels of aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) reduced with CN and increased with 2CN doses (p < 0.05). Alanine aminotransferase (ALT) and the atherogenic index remained similar among all treatments (p > 0.05). Hepatic vacuolation decreased with CN, but the 2CN dose produced mononuclear leucocyte infiltrate.

**Conclusions:** Although CN presented beneficial effects on the metabolism of rats, it also caused increased risk of liver damage.

**Keywords:** Plant extracts, Obesity, Toxicity, Pharmacology, Candlenut, Physiology

---

**Introduction**

Natural products and herbal medicines have been used indiscriminately for weight loss [1]. However, many of these compounds lack studies with scientific evidence of therapeutic potential or health risks [2]. The seed of *Aleurites moluccana* (l.) Willd (AM), also known as Kukui in Hawaii, candlenut (CN) in the US and Tuitui in the Cook Islands [3], has been used in the last years because of its medicinal properties.

The genus *Aleurites* is subdivided into *Aleurites montana*, *Aleurites trisperma*, *Aleurites cordata*, *Aleurites fordii* and *Aleurites moluccana*. The leaves of the plant *Aleurites moluccana* (AM) has been used to treat gastritis, fever, pain, diarrhea, asthma, and inflammation [4, 5] showing antibacterial [6] and antiviral effects [7]. AM's bark dichloromethane extract contains acetyl aleuritolic acid, atraric acid,
spruceanol, (5β,10α)-12-hydroxy-13-methoxy-8,11,13-podocarpatrien-3-one and sonderianol [8]. Besides, phytochemical composition of aqueous extract from AM seed present five mainly compounds: procyanidin dimer B1; 6-C-pentosyl-8-C-hexosyl apigenin; isovitexin; 6-C-pentosyl-8-C-pentosyl luteolin and neriifolin [9]. These compounds are known to present mostly antinociceptive and anti-inflammatory activity [8, 9]. On the other hand, the esters and saponins found in AM seeds can cause vomiting and diarrhea as adverse effects [10].

The seed of the plant has gained notoriety as a fast weight-loss agent, despite the scarcity of information about its pharmacological mechanisms of action. The weight-loss agent, despite the scarcity of information for body weight (grams)/naso-anal length (centimeters) × 1000. Values above 0.3 indicate obese animals [13]. The index was calculated on days 0, 30 and 58 of the experiment.

Methods

Animals

The experimental protocol was performed according to the Guide for the Use and Care of Laboratory Animals (ARRIVE guidelines for reporting in vivo experiments). The study was approved by the Ethics Committee on Animal Use of the Federal University of Lavras (UFLA) under protocol number CEUA 067/2016. Animals were treated in accordance with Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press). The sample size was determined to provide 80% power to recognize a significant difference of 20% among groups and a standard deviation of 15% with a 95% confidence for body weight (α = 0.05). Therefore, a sample size of five animals per group was required. Thirty adults male Wistar rats (Rattus norvegicus albinus) from the Central Animal Laboratory were used. The animals were healthy, with 90 days of age and an initial weight of 350 ± 28.7 g.

Firstly, we randomly distributed the animals into polypropylene cages (49 × 34 × 16 cm) and they underwent an acclimation period of 7 days under optimal conditions of temperature (22 ± 2 °C), humidity (45 ± 15%), and photoperiod (12/12 h light/dark cycles). Commercial feed and water were provided ad libitum. The commercial diet (Nuvilab CR-1®) contained 25.6% kcal protein, 62.6% kcal carbohydrate, 11.8% kcal lipid and 0.006% diet vitamin E.

Half of animals received a commercial diet for 30 days. The other half was induced to obesity by receiving a hypercaloric diet according to Oliveira et al [12]. The diet consisted of commercial feed (37%), roasted peanuts (25%), milk chocolate (25%) and cornstarch cookies (13%) at a 3:2:2:1 ratio. These ingredients were ground, mixed and offered in the form of pellets [12]. After 30 days of obesity induction, for the subsequent 28 days, CN was administered. During this period, the animals continued to receive their respective diet (Table 1). The schematic representation of the experimental design over time is shown in Fig. 1.

Obesity was determined by the Lee index: [cubic root of body weight (grams)/naso-anal length (centimeters) × 1000]. Values above 0.3 indicate obese animals [13]. The index was calculated on days 0, 30 and 58 of the experiment.

Administration of the candlenut

CN was administered by gavage using an endoscopic needle coupled to a 1-mL syringe. The seeds were macerated and homogenized in propylene glycol (PG), which was used as vehicle. Only the raw seed was used in the solution. The placebo groups received only propylene glycol (control).

The initial CN dose administered was calculated in proportion to the body weight of the animals, using as a reference the popular dose used for a person of 100 kg (kg). For human treatment with CN, it is recommended to ingest 1/8 of the seed in the first 8 days of treatment, and then 1/4 of the seed in the remaining days, independently of body weight [11]. For rats in the present experiment, the proportional dose was 0.2 mg/day for the first 8 days and 0.4 mg/day for the 20 subsequent days, respectively. Regarding the 2CN treatment, the groups

| Groups | Diets |
|--------|-------|
| 1      | Standard diet + propylene glycol |
| 2      | Standard diet + propylene glycol + CN |
| 3      | Standard diet + propylene glycol + 2CN |
| 4      | Cafeteria diet + propylene glycol |
| 5      | Cafeteria diet + propylene glycol + CN |
| 6      | Cafeteria diet + propylene glycol + 2CN |

CN: popular dose of candlenut (8 days with oral administration of 0.2 mg/kg followed by 20 days with oral doses of 0.4 mg/kg). 2CN: twice the popular dose of candlenut.
received 0.4 mg/day in the first 8 days and 0.8 mg/day on the other days, representing the doubled dose.

**Body weight and blood collection analysis**

During the experimental period, food consumption was measured daily, and the rats were weighed weekly. At the end of the experimental period (day 58), the animals were euthanized by cardiac puncture under anesthesia (Sodium thiopental, 50 mg/kg intraperitoneally) after fasting for 8 h. The concentrations of total cholesterol, high density lipoprotein cholesterol (HDL-c), triacylglycerols (TAG), glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyltransferase (GGT) were determined using commercial colorimetric/enzymatic kits (Labtest Diagnostica S/A®; Lagoa Santa, MG, Brazil). The low-density lipoprotein cholesterol (LDL-c) levels were calculated using the Friedewald formula, where LDL-c = Total Cholesterol – HDL-c – Triacylglycerols/5. The very low-density lipoprotein cholesterol (VLDL-c) values were measured using the following equation: VLDL-c = Triacylglycerols/5 [14]. The atherogenic index of plasma was calculated using the equation: log (TG)/(HDL-C), which is used as a significant predictor of atherosclerosis [15].

**Histopathological analysis**

The liver was collected and fixed in 10% buffered formaldehyde and was subsequently dehydrated in an increasing alcohol series, cleared in xylol, and embedded in paraffin to obtain 5-μm-thick slices as described previously [16]. Subsequently, the sections were stained with hematoxylin-eosin and analyzed under optical microscopy (Olympus CX31; Olympus, Tokyo, Japan) by a veterinarian pathologist blind to the treatments.

The microscopic lesions of the liver were classified regarding presence of cytoplasmic vacuolation, inflammatory infiltrate, necrosis, and vessel congestion. Besides, the lesions were classified as: incipient, when there were lesions in individualized cells; focal, when it occurred in a single point; multifocal, when it occurred at various points sparsely; focally extensive, when a considerable area of the parenchyma was affected; and diffuse, when all the parenchyma was affected. Additionally, liver tissue ratings were assigned according to the presence and/or degree of mononuclear infiltration as follows: No change; light--; discreet++; moderate++; severe++++.

**Carcass composition**

Carcasses were analyzed after removing skin, tail, paws, head, and viscera. The whole body was ground and homogenized to determine humidity, protein, collagen, fat, and mineral matter content. Near infrared reflectance (NIR) spectrometry was used, using the FoodScan™ NIR spectrophotometer (FOSS, Hillerod, Denmark) [17].

**CN fatty acid extraction by gas chromatography**

Macerated CN (10 g) was added to 80 mL of solvent in a flask, connected to a bulb condenser. The extraction process included the use of three solvents in order to determine the one with the best yield and extraction capacity. Extraction was conducted under reflux for 6 h from the time of boiling. Then, the sample was filtered and then rotaevaporated until solvent free [18].

CN humidity was determined by adding approximately 3 g of the pulp and 80 mL of cyclohexane in a round-bottomed flask, which was coupled to the Dean stark apparatus. The flask was heated and after 2 h, the volume of water present in the plant material was quantified [19]. The CN oil yield was calculated and expressed as the weight of oil divided by the weight of the material on a moisture-free basis (MFB).
Next, 100 mg of CN oil, 2 mL of hexane (HPLC grade) and 0.2 mL of 2 mol/L KOH methanolic solution were added to a test tube. Thereafter, the tube was vortexed for 30 s. After stirring, 3 mL of saturated sodium chloride solution was added to the tube. Subsequently, the tube was allowed to stand for phase separation. The upper layer was removed for chromatographic analysis [18, 20].

Fatty acid (FA) composition was determined on a gas chromatograph (model Shimadzu CG-17a). We used a SP 2560 column 100-mm long by 0.25 mm in internal diameter and 0.2 µm thickness of the liquid-phase film with a flame ionization detector at 260 °C, split injector (1:20 ratio) at 260 °C with an initial oven temperature of 140 °C for 5 min. As a qualitative analysis, the chromatogram obtained by the injection of the samples was compared with that obtained for the PUFA standard, with integration of the peaks. Fatty acids were identified by comparison of retention times.

**Statistical analysis**
The study design was completely randomized with a 2 × 3 factorial arrangement (obese or non-obese and three treatments: without CN, CN and 2CN). The data were subjected to analysis of variance (two-way ANOVA), and the means were compared by Tukey’s test at 5%. The Lee index values along time were compared using three-way-ANOVA. The analyses were performed using the SAS statistical program (1996).

**Results**
The major fatty acid found on candlenut oil were methyl palmitate, methyl palmitoleate, linolelaidic acid methyl ester and methyl arachidate. Besides, the trans-9-elaidic acid methyl ester was the only component found in all extraction protocols using three different solvents (Table 2; Fig. 2).

After 30 days receiving cafeteria-diet, all animals from groups 4 to 6 were metabolically obese (Lee index > 0.3). Lee index of Animals from groups 1 to 3 presented values < 0.3. After 28 days of CN treatment, the obese animals showed a significant reduction of the Lee index, reaching values under the obesity range (Fig. 3).

Weight gain was higher in the animals that consumed the cafeteria diet. CN and 2CN ingestion significantly decreased weight gain for 28 days (p < 0.05) both in the cafeteria and the standard diet groups (Fig. 4). The amount of consumed feeding (in grams) was not different in groups receiving standard and cafeteria diets (p > 0.05), although cafeteria diet was hypercaloric. CN ingestion decreased food consumption also for both standard and cafeteria groups (p < 0.05), with a dose-dependent effect for the cafeteria group only (Fig. 4).

Cafeteria-diet increased collagen, fat and mineral matter while decreased protein and humidity in carcass. CN ingestion increased collagen, humidity and protein content while reduced fat percentage in carcass (p < 0.05) (Fig. 5). Significant interaction existed between diet and CN ingestion for fat and humidity parameters (p < 0.05).

Concerning plasma lipoproteins, the concentrations of triacylglycerols, total cholesterol and fractions were generally increased by cafeteria diet and reduced by CN (p < 0.05) (Fig. 6). Besides, we also found significant

| Number | Identified fatty acids | Retention time | Hexane | Methanol | Centrifuged methanol % of Area |
|--------|------------------------|----------------|--------|----------|-------------------------------|
| 1      | Methyl hexanoate       | 8.320          | 1.3904 | –        | –                             |
| 2      | Methyl octanoate       | 9.416          | 0.1678 | –        | –                             |
| 3      | Methyl myristate       | 17.457         | –      | –        | 0.1755                        |
| 4      | Methyl palmitate       | 20.998         | 21.2427| 19.8421  | –                             |
| 5      | Methyl palmitoleate    | 21.046         | –      | –        | 37.4686                       |
| 6      | Methyl heptadecanoate  | 22.167         | 0.3760 | –        | 0.4484                        |
| 7      | Cis-10-heptadecenoic acid methyl ester | 22.761 | – | – | 0.1612 |
| 8      | Methyl stearate        | 23.789         | –      | –        | 0.085                         |
| 9      | Trans-9-elaic acid methyl ester | 24.496 | 5.9588 | 3.8231 | 10.3682 |
| 10     | Linolelaidic acid methylest | 25.348 | 40.3870 | 33.6412 | –                             |
| 11     | Methyl arachidate      | 26.842         | –      | 41.6657  | 48.0404                       |
| 12     | Methyl eicosanoate     | 27.679         | –      | –        | 2.1375                        |
| 13     | Cis-11,14,17-eicosatrienoic acid methyl ester | 30.657 | – | – | 1.1152 |
reductions in blood glucose levels (p < 0.05). Blood glucose levels of obese rats reaching values similar to the groups receiving standard diet (Fig. 6). Atherogenic index were similar between diets and among treatments (Fig. 6). Liver enzymes AST and GGT, decreased when the CN dose was administered. However, their values increased (p < 0.05) in groups receiving 2CN treatment (Fig. 7). ALT enzyme concentration was similar between diets and among treatments (Fig. 7).

Rats receiving cafeteria diet presented hepatic vacuolation, and the degree of degeneration decreased slightly with CN and 2CN treatments. However, a mononuclear infiltrate was found in the liver of animals that consumed candlenut, especially 2CN. Ballooning and fibrosis were not reported in the histopathological evaluation of the present study (Fig. 8). Both placebo groups (cafeteria and control diets) presented discrete to mild (+/++) mononuclear infiltrate, while CN and 2CN groups had moderate to severe in-filtrates (+++/+++++).

**Discussion**

The results of the present study demonstrated a reduction in weight gain and potential improvement of blood biochemical parameters of obese (and non-obese) rats that consumed candlenut for 28 days. In general, the effects were positive, with the reduction of the carcass fat; total cholesterol and its fractions; and also, a reduction of blood glucose levels. However, some negative
In the present study, obesity was induced in Wistar rats using the cafeteria diet. At the end of 30 days, induced obesity was confirmed by the Lee index [13]. The said model is efficient in a short time [21]. The cafeteria diet is composed of more palatable foods that trigger hyperphagia, leading to increased body weight, hyper-adiposity and hyperglycemia [22], as observed in the placebo group (G4). Additionally, high consumption of sucrose and fat stimulates the release of dopamine, reducing short-term stress, which may also contribute to body weight gain [23].

Animals that ingested candlenut had a reduction in body weight, taking Lee index values below 0.3 after 28 days of treatment. This fact demonstrated that candlenut was able to reduce obesity and control weight gain in rats. Similar properties were found in a study carried out by Pedrosa et al., [24] using Aleurites moluccana leaf extracts. AM seed presents highest concentrations of phenolic compounds, tannins and flavonoids [9] which are phytochemicals with recognized effect to promote weight loss [25–27]. Furthermore, the presence of α- and β-amyrenone in the AM seed is associated with the inhibition of enzymes responsible for lipid and carbohydrate

effects were observed, such as HDL-c reduction and increased AST and GGT levels especially when CN dose was doubled.
Fig. 5 (See legend on previous page.)
absorption [28, 29]. The mechanism of action of CN derivatives remains unclear. The loss of fluids and electrolytes is likely caused by its laxative and diuretic effects, and the report of diarrhea is common [11]. In the present study, diarrhea was not observed. However, such findings should be evaluated with caution because the animals were kept under controlled conditions, without interference of physical activity, other drugs, alcoholic beverages, anabolic substances, among other factors, hindering direct extrapolations to humans.

The carcasses of rats that consumed candlenut presented lower concentrations of fat and dry matter, which were concentration dependent—i.e., at the higher dose (2CN), both the animals feeding on the standard diet and cafeteria diet showed more intense reduction (p < 0.05). Such results confirm the ability of the seed to affect body fat accumulation in rats. Besides, these results may be related to the fatty acids present in the candlenut structure, especially linoleic acid (ω-6) and palmitoleic acids (ω-7). Linoleic acid acts as an intra-cellular signal to induce the transcription of genes involved in lipid oxidation and inhibits the expression of genes involved in lipogenesis [30] by binding to the PPAR-α nuclear receptors (peroxisome proliferator-activated receptors) [31–34]. Additionally, it acts by inhibiting cholesterol biosynthesis by inhibiting the actions of SREBP-1 and SREBP-2 (sterol-regulatory element-binding proteins) [35]. Furthermore, palmitoleic acid acts as an adipokine, a signaling molecule produced by adipocytes, stimulating the effects of insulin on the muscles and reducing hepatic steatosis [36]. These mechanisms can also explain the effects of CN in reducing total cholesterol and LDL-c, HDL-c, VLDL-c fractions, and triglycerides.

In obese animals, it was possible to observe that the 2CN dose reduced LDL-c (p < 0.05), without significantly altering HDL-c. These results differ from those found previously [24] for Aleurites moluccana leaves, who found a reduction of LDL-c values and an increase in HDL-c levels. The different behaviors of LDL-c and HDL-c parameters in response to candlenut indicate a variation in the distribution of cholesterol lipoproteins, perhaps with increased hepatic receptors that catabolize LDL-c [37]. Indeed, our results demonstrated the hypocholesterolemic properties of CN. Reduced LDL-c levels are associated to a reduced atherosclerosis risk [38]. The reduction in HDL-c levels is not a desirable outcome, as this lipoprotein is related to lower cholesterol deposition, and consequently, less chance of atherosclerotic plaque formation in the vascular endothelium [39].

In this study, blood glucose levels were also significantly reduced in both obese and non-obese animals from groups that received CN. Hyperglycemia is commonly found in obese individuals and represent a sign of insulin resistance [40]. However, the reduction in glycemia observed in the present study in non-obese normoglycemic animals highlights another undesirable effect of CN, since the lower availability of circulating glucose affects the proper functioning of several organ systems. Thus, hypocholesterolemia caused by CN associated to blood glucose decrease, may be related to alterations in the intestinal absorption of nutrients, compromising the availability of circulating energy.

Regarding the liver enzymes AST and GGT, there was a reduction of their plasma concentrations in the obese rats that received the low CN dose, but the AST values in creased with the 2CN dose—even when compared with both non-obese and obese placebo groups. Increased levels of AST may be related to liver damage or disease [41]. In addition, this outcome may be possibly due to hepatotoxicity induced by high doses of CN. Higher transaminases levels are associated with hepatic toxic events such as cell membrane disruption, mitochondrial dysfunction, oxidative stress, and recruited inflammatory cells [42]. To corroborate the suspicion of liver damage, we observed hepatic mononuclear infiltrate appeared in the samples from animals that ingested the seed at the different doses, indicating the onset of hepatic inflammation. Although liver damage observed on AST may be considered small, it appeared after only a short period of 28 days of CN treatment. We decided to evaluate 28 days therapy due to the normal popular consumption for this medicinal plant. However, abuse is not uncommon and deserves proper investigation. Future studies should evaluate chronic consumption of CN for longer periods.

Regarding the liver enzymes AST and GGT, there was a reduction of their plasma concentrations in the obese rats that received the CN dose (p < 0.05), but the GGT values in creased with the 2CN dose (p < 0.05). Additionally, rats consuming a cafeteria diet had hepatic vacuolation, which was reduced slightly with the CN
Fig. 6 (See legend on previous page.)
and 2CN treatments, probably due to the reduction in body fat caused by the seed. Hepatocyte vacuolation is associated with non-alcohol-related fatty liver disease [43]. These results demonstrate that consumption of CN attenuated the accumulation of lipids in the liver. However, a hepatic mononuclear infiltrate appeared in the samples from animals that ingested the seed at the different doses, indicating the onset of hepatic inflammation. There is a shortage of data on the toxicity of Euphorbiaceae species in rats and humans, but these species are potentially toxic, as observed in other species [44]. One should consider that humans often associate candlenut ingestion with food restriction, overtraining, other drugs, alcoholic beverages, anabolic substances, which can overload liver function leading to higher probability of adverse effects. Studies
at higher doses or for longer periods are necessary to assess the potential toxicity of the seed.

In summary, candlenut presented favorable results regarding weight control, body fat, blood lipoproteins and glucose levels. However, it is of paramount importance to note that these effects were achieved in Wistar rats under controlled laboratory conditions. Future studies are encouraged in order to elucidate toxicological aspects elicited in the pre-sent study. In humans, the combination of natural products, excess physical activity and/or other drugs is common, and the potential associated effects are still unknown. Additionally, the seed has been used by the population worldwide, with reports of toxicity and death [11]. Indiscriminate use of candlenut, whether at high amounts or for a pro-longed time, is reported to cause feelings of discomfort and nausea. These symptoms may be followed by vomiting, abdominal pain, diarrhea, dehydration and changes in the heart rate. Future studies analyzing the effects of the consumption of the seed on the blood pressure and molecular pathways are necessary to ensure its safe use. Additionally, it is interesting that future studies evaluate the effects of CN consumption on hormones involved with food ingestion control, such as leptin.

In conclusion, CN consumption for 28 days reduced body weight, carcass fat, plasma lipoproteins, blood glucose levels and the degree of vacuolation in Wistar rats. Higher doses were associated to mononuclear liver infiltrate and increased AST and GGT levels, indicating the risk of liver overload and damage.

Acknowledgements

The authors gratefully acknowledge FAPEMIG (Research Support Foundation of Minas Gerais), CAPES (Coordination for the Improvement of Higher Education Personnel) and CNPq (National Council for Research and Development) for their financial support.

Author contributions

MCBR, PRR: Methodology, data curation, writing—original draft. VOS, DACSR, TPS, MGC, VP: Data Curation, Resources, Writing—Review & Editing, Visualization. JNS: Data Curation, Writing—Review & Editing. EFA: Data Curation, Writing—Review & Editing, Visualization. RMM, LJP: Conceptualization, Resources, Writing—Review & Editing, Visualization, Project administration. LJP, MGC and RMM contributed to the conceptualization and study design. MCBR, PRR, VOS, DACSR, TPS, FRs, MGC, JNS, EFA, VP, RRM and LJP contributed to the acquisition and interpretation of the data. MCBR, VOS, DACSR, TPS and FRs performed formal analysis. MCBR, PRR and VOS drafted the manuscript. MGC, JNS, EFA, VP, RRM and LJP critically revised the manuscript. LJP, RRM and MGC supervised the study. All authors contributed substantially to the execution of this study and approved the final version of this manuscript. All authors read and approved the final manuscript.

Funding

We thank to National Council for Scientific and Technological Development (CNPq), the Higher Education Personnel Improvement Coordination (CAPES) and the Minas Gerais State Research Support Foundation (FAPEMIG).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.
