Investigation of the antihyperlipidemic property of *Raphia hookeri* mesocarp oil in high-lard diet (HLD) fed rats

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Abstract

**Background:** Dyslipidemia is an important risk factor cardiovascular disease. The mesocarp of *R. hookeri* has been shown to resemble that of palm oil in term oil quality, with a large quantity of linoleic acid (47.3%) followed by palmitic acid (32.3%), oleic acid (13.7%) and alpha-linolenic acid (1.1%). The present investigation aimed to evaluate the antihyperlipidemic property of crude *Raphia hookeri* mesocarp oil in male *Wistar* albino rats fed with high-lard diet (HLD).

**Methods:** Crude *Raphia hookeri* mesocarp oil (1 mL and 2 mL per kg of body weight respectively), and olive oil as control (1 mL and 2 mL per kg of body weight respectively) were administered orally by gavage and lasted 28 days along with high-lard diet (HLD). Biochemical parameters of lipids profile were estimated in plasma after the treatment.

**Results:** Untreated rats fed with HLD showed profound alterations of the lipid profile and doses of *Raphia hookeri* mesocarp and olive oil showed a significant (P < .05) reduction in elevated TC (total cholesterol), TG (triglycerides), LDL (low density lipoprotein), and VLDL (very low density lipoprotein) levels and increased HDL (high density lipoprotein) level. Best effects were observed with the dose of 1 mL/kg in TC, TG, and VLDL levels, and 2 mL/kg in HDL level. Doses of olive oil were shown to be most effective in increasing the HDL level.

**Conclusion:** The crude oil from *Raphia hookeri* mesocarps exhibited an antihyperlipidemic effect on albino rats fed with high-lard diet. Its effect is similar to that of olive oil and may possibly be used to prevent dyslipidemia.

**Keywords:** *Raphia hookeri*; oil rats; antihyperlipidemic; high lard diet

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Background

Cardiovascular diseases (CVDs) remain a leading cause of death and morbidity, ranking first globally [1]. According to the World Health Organization (WHO), ~17.9 million people die from CVDs each year-representing 31% of all global deaths, over 75% occurring in low- and middle-income countries [1]. The most common, described in approximately 85% of all CVD deaths, are heart attack (~7.4 million deaths) and stroke (~6.7 million deaths) [1]. Additionally, cardiovascular disorders are shown to alter the life prognosis in individuals when coexisting with other health problems of global significance, such as acute respiratory infections [2,3]. A recent meta-analysis supported this evidence in individuals suffering from the novel coronavirus (COVID-19) pandemic [4].

The management of CVDs is expensive, and drugs used are generally associated with adverse side effects. Evidence shows the effectiveness of healthy diet patterns and lifestyle for the prevention of cardiovascular disease and it is believed that an adequately balanced diet plays a key role in this prevention [5]. That said, a particularly crucial role is ascribed to bioactive compounds, such as dietary fiber, sterols, vitamins, probiotics and prebiotics, polyphenols, and fatty acids [6]. An important part of a healthier diet, with the example of the traditional so-called “Mediterranean diet”, is believed to be an increased intake of polyunsaturated fatty acids (PUFAs) and polyphenols [7]. Several studies have suggested that PUFAs, alpha-linolenic acid (n-3) and linoleic acid (n-6) consumption, may be associated with low risk of cardiovascular disorders [8,9,10]. Additionally, in a recently published study carried out on rats, a seed oil with high linoleic acid content was shown to significantly improve the blood lipid profile [11]. Plant and seed oils are major sources of linoleic acid [12], but alpha-linolenic acid can be found in large amounts in some plant oils [13].

*Raphia hookeri* (RH) commonly called raffia palm is a family member of *Palmaceae*. It is a fruit plant commonly found in tropical and sub-tropical regions around the world. The mesocarp of *R. hookeri* has been shown to resemble that of palm oil not only in term of lipid content (40-60%), but also in term oil quality, with a large quantity of linoleic acid (47.3%) followed by palmitic acid and oleic acid, 32.3% and 13.7% respectively, and 1.1% of alpha-linolenic acid [14]. Additionally, this mesocarp is yellow-like colored and its oil, red-like colored; and it is believed that yellow to red-like colored plants are good sources of a group of fat-soluble compounds, called carotenoids which have antioxidant properties [7].

Based on the fatty acid composition of this mesocarp oil, we hypothesized that *Raphia hookeri* mesocarp oil might help in preventing from some cardiovascular risk factors, thereby altering blood lipid concentrations. Therefore, this study aimed to evaluate the capacity of *Raphia hookeri* mesocarp oil to alter the lipid profile.

Methods

**Plant Material**

*Raphia hookeri* fruits were collected in Mbaleveng, Dschang, Cameroon from April to June 2018 and identified in National Herbarium under the voucher number: 38372/HNC. The mesocarps were separated and cut into small pieces using a rustproof knife cleaned and dried in an air-dried oven at 45 °C for 48 hours.

**Extraction of Crude Raphia hookeri mesocarp oil**

Dried mesocarps were crushed and powdered using a mechanical grinder. Lipids were extracted by mixing every 200g of the powder from dried mesocarps into 500 ml of hexane, with regularly shake for 48 hours at room temperature. The extract was filtered using a Whatman N°1 filter paper and the solvent removed using rotary evaporation under reduced pressure at 50 °C. Crude *R. hookeri* mesocarp oil obtained was weighed the extraction yield estimated and stored at −18°C prior to further analysis.

**Experimental Animals and Experimental design**

Thirty male Wistar albino rats weighing 150-200 g were used for this study. All animals were individually isolated in cages under standard laboratory conditions with a 12-hour day dark-light cycle and randomly divided into six groups (n = 5). Respective groups were initially fed for six weeks with normal laboratory and high-fat diets. After this period, the group 1, normal untreated fed with normal laboratory diet served as normal control and was daily provided with water by gavage. The five other groups fed with high-lard diet were organized as follow: group 2 served as hyperlipidemic control and was daily provided with water, treated groups 3 and 4 with crude *Raphia hookeri* mesocarp oil (1 mL and 2 mL per kg of body weight respectively), and treated groups 5 and 6 with olive oil (1 mL and 2 mL per kg of body weight respectively). Treatments were administered orally by gavage and lasted 28 days. All animals were allowed to drink water at *bibium*. Food intake and body weight were followed throughout the experimentation. On day 28, twelve hours after the last oral administration, blood samples were collected from heart aorta under light anesthesia with chloroform, and animals killed before the extraction of organs (liver, heart, kidney, and spleen). The serum was separated from the blood by centrifugation at 3000 rpm for 15 minutes and the sera were stored in Eppendorfs at −20°C for further biochemical analysis.

All the experimental procedures were conducted after the approval by the Institutional Review Board of the University of Dschang, Cameroon, and reviewed at the end by the same organ.

**High-Lard Diet Protocol**

The lard was purchased from a local market in Dschang, Cameroon, melted at high temperature into unrefined palm oil in a ratio 5:3 (w/v), cooled at room temperature and filtered. To obtain the high-lard diet, the described protocol [15] was followed.

**Determination of Oxidation State and Stability.** The peroxide value was determined by spectrophotometry using the IDF standard method 74A: 1991 and expressed in milliequivalent of oxygen per kilogram (meq O₂/kg) of fat. The iodine value was determined using the Association of Official Analytical Chemist (AOCS) official method [16] and expressed in gram of iodine fixed per hundred grams (g I₂/100 g) of fat. The thiorbarbituric acid value was determined by the described method [17] and expressed in part per million (ppm). The acid value was expressed as milligram of KOH per gram (mg KOH/g) of fat.

**Estimation of Lipid Profile Level and Atherogenic Indexes**

The total cholesterol (TC), triglycerides (TG) and high-density lipoprotein (HDL) were determined using enzymatic kits (CHRONOLAB) and low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were calculated by Friedewalds formula.
as given below. The atherogenic indexes (AI) were calculated as well.

\[ \text{VLDL} = \frac{\text{TG}}{5} \quad \text{LDL} = \frac{\text{TC} - (\text{HDL} + \text{VLDL})}{\text{AI}} = \frac{\text{TC}}{\text{HDL}} \]

**Statistical Analysis**

IBM SPSS Statistics 21 was used to perform all analyses. Data are expressed as mean ± standard deviation (SD) and evaluated by one-way analysis of variance (ANOVA) followed by LSD tests for multiple comparisons and values of P < .05 were considered statistically significant.

**Results**

**Extraction yield, Oxidation state and stability**

The extraction yield of crude *Raphia hookeri* mesocarp oil was approximately 63.74% and is shown together with oxidation state and stability values in Table 1. These values matched with standard values concerning crude vegetable oil.

**Food intake, growth rate relative weight of organs**

The effect of *Raphia hookeri* mesocarp oil on daily food intake (Figure 1) and growth rate per day (Figure 2) was assessed. Groups fed with high-lard diet showed a slight reduction in daily food intake compared to the normal untreated group feeding normal laboratory diet. The same result was observed with their growth rates. Groups fed with HLD showed a reduction in their daily growth rates compared to the normal untreated group. In Figure 3 is shown the effect of *R. hookeri* mesocarp oil on the relative weight of organs and it can be observed that lard high-fat diet did not affect vital organs.

**Effect of Raphia hookeri mesocarp oil on lipid alterations**

The untreated group fed with HLD showed profound alterations of the lipid profile (Figures 4 and 5). Doses of *Raphia hookeri* mesocarp and olive oils showed a significant (P < .05) reduction in elevated TC, TG, LDL, and VLDL levels and increased HDL level. An exception was shown at the dose of 2 mL/kg of olive oil. Best results were observed with the dose of 1 mL/kg in TC, TG, and VLDL levels, and 2 mL/kg in HDL level. Different doses of olive oil were shown to be most effective in increasing HDL level. Additionally, doses of *Raphia hookeri* mesocarp and olive oils have shown significant (P < .05) amelioration of the atherogenic effect in high-lard diet fed rats (Figure 6).

**Discussion**

High-fat diet (HFD) is generally given to rats to elicit obesity and dyslipidemia, and mimic feeding style more common in western countries [17]. In several studies, high-fat diet with lard (high-lard diet) is generally associated with body weight gain in rats, even after a short period [18, 19]. However, these evidences were not supported in the current study. High-lard diet tended to slow-down the growth rate of rats. This may be explained by the reduction in food intake observed during the experimentation, suggesting that the heated treatment performed on the lard when melting it into palm oil may have contributed to spoiling the diet, affecting organoleptic properties.

In general, lipid metabolism imbalance also known as dyslipidemia, often result from interactions between unhealthy dietary patterns and genetics [20]. Dyslipidemia is a condition of having abnormal blood lipid concentrations. It is characterized by alterations in lipid profile with increased total cholesterol (TC), triglycerides (TG) and low-density lipoproteins (LDL) and decreased high-density lipoproteins (HDL) [21].

The elevated total cholesterol (TC) is one of the major risk factors of coronary heart disease (CHD). In dyslipidemias, hepatic LDL receptors become incompetent, LDL circulating through the body, its degree of oxidation increases and contact with vascular endothelium is prolonged, increasing by the way the atherogenic effect [22]. Atherosclerosis is the leading cause of fatal cardiovascular events worldwide. Atherosclerosis derived from dyslipidemia is characterized by deposits of atheroma plaques on the intestinal walls of medium and large arteries, with thickening of the arterial and loss of elasticity [23]. Hence, control of blood lipid concentrations is beneficial in cardiovascular disease prevention. The *Raphia hookeri* mesocarp oil showed a reduction in atherogenic indexes and improved blood lipid concentrations. The blood lipid lowering efficacy of doses of *Raphia hookeri* mesocarp oil could be beneficial in preventing cardiovascular events.

Dietary and healthy lifestyle are considered a practical approach to cardiovascular disease prevention. The main benefits of the Mediterranean diet in CVD have been associated with a better control of risk factors to improve blood pressure, lipid profile, glucose metabolism and others [24]. Virgin olive oil (VOO) is the primary fat source in the Mediterranean diet. VOO is rich in polyphenols, namely oleuropein and monounsaturated fatty acids-MUFA [25]. Cardioprotective effect of MUFA rich diet resulted from an enhancement of HDL and decrease of LDL levels [26]. In a randomized crossover study, LDL-cholesterol decreased after consuming a diet enriched with VOO [27]. Therefore, as *Raphia hookeri* mesocarp oil showed similar beneficial effect as that of olive oil used as a control, this may be attributed to its composition. However, *Raphia hookeri* mesocarp oil has been shown to be different in fatty acid composition compared to olive oil [13].

Indeed, it has been shown that abundant fatty acid in *Raphia hookeri* mesocarp oil was linoleic acid (LA), a polyunsaturated fatty acid [13]. LA has been reported to be associated with lowering LDL, blood pressure and insulin resistance, all important risk factors of atherosclerosis [28]. However, concerns have been raised that LA confers a higher risk of vascular disease due to its role as a precursor of arachidonic acid considered as a precursor of pro-inflammatory and pro-thrombic compounds, namely eicosanoids [29]. Nevertheless, there is actually some indications that LA might possess anti-inflammatory effects [12, 30]. Indeed, several studies have suggested that long-chain omega 3 PUFAs and LA may be associated with a lower risk of coronary heart disease [8,10].

Mesocarp lipids are generally of two types: those with high lipids content, rich in phospholipids and glycolipids, and those with high lipids content, rich in triglycerides. Lipids content of mature fruit mesocarps from which palm oil is extracted are often high as 70% [31]. Mesocarps of Raphia fruits resemble that of the palm oil in term of lipids content (40-60%) and composition [13]. The extraction yield of *Raphia hookeri* mesocarp oil (63.94%) was similar to that reported [13]. Generally, fats and oils are unstable because of their chemical composition and sensitivity to oxidation. This oxidation process results in an undesirable flavor known as rancid, responsible for consumer’s rejection. To determine the oxidative state of fats and
oils, iodine, peroxide and thiobarbituric acid values are commonly assessed [32]. The iodine value measures the unsaturated bonds in fats and oils. That said, high iodine value is proportional to the unsaturated fatty acid content of a fat or oil. The iodine value of crude *Raphia hookeri* mesocarp oil was slightly high (60.1 ± 0.3 g I₂/100g) indicating its potential source of unsaturated fatty acids. This value resembles that of palm oil (44-58 g I₂/100g) and is less than that of olive oil (75-94 g I₂/100g) [33]. The iodine value usually decreases with time as the oxidation process advances. The peroxide value estimates the primary oxidation state of lipids while the thiobarbituric acid value informs about the secondary oxidation products responsible for rancid in lipids. In general, fresh oils have a peroxide value of less than 10meq O₂/kg [32], accepted range for crude oils being 0-15 meq O₂/kg [34]. The peroxide and thiobarbituric acid values (8.78 ± 0.3 meq O₂/Kg and 2.38 ± 0.3 ppm, respectively) were matching with standards concerning crude vegetable oils.

**Figure 1.** Effect of *Raphia hookeri* mesocarp oil on food intake in high-lard diet induced hyperlipidemic rats.
Results are expressed as mean ± SD (n = 5).

**Figure 2.** Effect of *Raphia hookeri* mesocarp oil on growth rate in high-lard diet induced hyperlipidemic rats.
Results are expressed as mean ± SD (n = 5).

**Figure 3.** Effect of *Raphia hookeri* mesocarp oil on some organs in high-lard diet induced hyperlipidemic rats.
Results are expressed as mean ± SD (n = 5).

**Figure 4.** Effect of *Raphia hookeri* mesocarp oil on total cholesterol (TC) and total triglycerides (TG) in high-lard diet induced hyperlipidemic rats.
Results are expressed as mean ± SD (n = 5). a,b P < .05: hyperlipidemic group compared with other groups.

**Figure 5.** Effect of *Raphia hookeri* mesocarp oil on HDL, LDL, and VLDL cholesterol in high-lard diet induced hyperlipidemic rats.
Results are expressed as mean ± SD (n = 5). a,b P < .05: hyperlipidemic group compared with other groups.

**Figure 6.** Effect of *Raphia hookeri* mesocarp oil on atherogenic index (AI) in high-lard diet induced hyperlipidemic rats.
Results are expressed as mean ± SD (n = 5). a,b P < .05: hyperlipidemic group compared with other groups.

| Table 1. Oxidation state and stability of *Raphia hookeri* mesocarp oil |
|-------------------|------------------|
| Tests             | Values           |
| Iodine value      | 60.1 ± 0.3 g I₂/100g |
| Peroxide value    | 8.78 ± 0.3 meq O₂/kg |
| Thioabarbituric acid value | 2.38 ± 0.3 ppm |
| Acid value        | 6.94 ± 0.3 mg KOH/g |
| Extraction yield  | 63.94%           |

**Conclusion**
The present findings demonstrated that the crude oil from Raphia hookeri mesocarp exhibited an antihyperlipidemic effect on rats fed with high-lard diet. The antihyperlipidemic effect of Raphia hookeri mesocarp crude oil is similar to olive oil used as the control, and the activity observed may due to the presence of bioactive compounds such as linoleic acid (ω3 PUFA) and fat-soluble polyphenols, which may indirectly help to prevent lipid profile alteration and LDL oxidation.

**Abbreviations**

- HLD: high-lard diet
- TG: triglycerides
- CT: total cholesterol
- LDL: low density lipoprotein
- HDL: high density lipoprotein
- VLDL: very low-density lipoprotein
- CVD: cardiovascular diseases
- PUFA: polyunsaturated fatty acids
- MUFA: monounsaturated fatty acids

**Authors’ Contribution**

APNK defined intellectual content, drafted and reviewed the manuscript; ABDEL AZIZ NJONGUMBE carried out the study; PTN performed data analysis and statistical analysis; HTN did the data acquisition.

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**Conflict of interest**

The authors declare no competing interest

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