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Full Length Research Paper

Serum lipid profile based on the prandial state among adult subjects

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This study was aimed to access the variations in serum lipid parameters based on the prandial state of adult patients. Through a cross-sectional descriptive and analytical study conducted in Parakou (Benin), from February 1 to May 1, 2020, 100 adult subjects (average age 31.21±12.36 years) were selected with systematic census. After ethical advice, a first sample was taken on empty-bellied adult subjects. Then, three additional samples were respectively taken two hours, four hours, and six hours after these subjects had eaten a meal of their choice. Serum lipid parameters were measured through enzymatic colorimetric methods. The average values of fasting lipid parameters in g/L were: Total cholesterol (1.48±0.35), HDL cholesterol (0.49±0.14), LDL cholesterol (0.84±0.36) and triglycerides (0.63±0.34). The comparison of the fasting and postprandial lipid parameters values is presented as follow: Triglyceridemia was significantly higher 2 h (P=0.000) and 4 h after meal (P=0.001). Only one type of food consumed was associated with mixed hyperlipidemia at six hours postprandial state (P=0.022). It may therefore be concluded that consumed foods do not cause most dyslipidemias 6 h in the postprandial state. During the screening for dyslipidemias in adults, the lipid profile can therefore be measured after six hours postprandial state.

Key words: Lipid profile, fasting, prandial state, adults.

INTRODUCTION

Serum lipid profile is commonly determined for the cardiovascular risk prediction. It is a routine medical examination that helps perform the screening of dyslipidemias (Nigam, 2011). Dyslipidemia is an important risk factor for coronary artery disease and stroke.

The joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine recommend several conditions for lipid test: i) non-fasting in most patients, including initial lipid profile testing in any patient, for cardiovascular risk assessment, patients admitted with acute coronary syndrome, in children, if preferred by the patient, in diabetic patients (due to hypoglycaemic risk), in the elderly and patients on stable drug therapy; ii) fasting can sometimes be required if non-fasting triglycerides >5 mmol/L (440 mg/dL), known hypertriglyceridaemia followed in lipid clinic, recovering from hypertriglyceridaemic pancreatitis, starting

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medications that cause severe hypertriglyceridaemia, additional laboratory tests are requested that require fasting or morning samples (Catapano et al., 2016).

For long, fasting blood samples have been considered as a standard for the measurement of lipid parameters after 8 to 12 h fasting (Stone et al., 2014; Driver et al., 2016). In their physiology, human beings do not adhere to that fasting time limit on a daily basis. Many studies have reported that the benefits of fasting lipid tests are not higher than those done in a non-fasting state (Nordestgaard, 2017; Pati and Singh, 2017; Scartezini et al., 2017). Well-conducted prospective studies found similar associations with cardiovascular risk by using lipid profile in the fasted state as well as in the non-fasting state (Mora et al., 2008; Langsted et al., 2008; Di Angelantonio et al., 2009; Doran et al., 2014).

To date, there is no scientific evidence explaining why fasting should be better than non-fasting during the lipid test assessment for the prediction of cardiovascular risk. Several clear benefits of non-fasting rather than fasting samples for lipid parameters' determination, may be mentioned: (i) blood collection in laboratory is simplified; (ii) the inconveniences related to fasting are avoided for the benefit of the patients; (iii) the risk for hypoglycemia due to fasting is minimized for subjects with diabetes (Anderson et al., 2016; Catapano et al., 2016; Jellinger et al., 2017; Scartezini et al., 2017).

Lipid parameters' determination according to the prandial state has been reported in literature. No difference was observed between fasting and non-fasting values of total cholesterol (TC), HDL cholesterol (HDL-C), and LDL cholesterol (LDL-C) whereas triglyceridemia (TG) was significantly higher in the postprandial state among diabetic patients (Gupta et al., 2016). A substantial increase in postprandial triglyceridemia among subjects with dyslipidemia on drug therapy and also among untreated subjects was found (Abdel-Aziza et al., 2017). The ASCOT-LLA study carried out on 8270 patients in the postprandial state, revealed a moderate increase in triglyceridemia and marginal change in HDL-C, LDL-C and TC compared to fasting subjects (Mora et al., 2019). In an observational study, non-fasting triglyceridemia is approximately 20% higher on average than the one measured in the fasted state; although the magnitude of the difference is subject to substantial interpatient variability (Rahman et al., 2018).

Many countries are currently revising their guidelines, seeking a consensus on lipid profile measurement for the prediction of cardiovascular risk in the non-fasting state (Anderson et al., 2016; Catapano et al., 2016; Jellinger et al., 2017; Scartezini et al., 2017). In Africa, and more particularly in Benin, lipid profile measurement in the fasted state is always adopted despite frequency of examination request for the exploration of cardiovascular diseases (CVDs). In Benin, to the best of our knowledge, no study has been conducted in order to reduce patients' constraints for the performance of lipid profile test, to get results in the physiological condition, and to assist the clinician in making a quick decision. This research work aimed to investigate variations in serum lipid parameters based on the prandial state among adult subjects in order to consider doing non-fasting lipid profile test.

MATERIALS AND METHODS

Type and period of study

This research work was a descriptive cross-sectional study with analytical purpose; the data used were collected over a three-month period running from February 1 to May 1, 2020.

Study target population

It consisted of adult subjects from both sexes living in the city of Parakou (Republic of Benin). This study included subjects, healthy or not, aged 18 years and more, who gave their writing informed consent to participate to the study. Pregnant and breastfeeding women were excluded.

Sampling

The authors did a systematic census of all adult subjects who volunteered to participate in this study during the data collection period, and meeting our inclusion criteria, in Parakou Teaching Hospital. This study involved 100 adult subjects.

Study variables

The dependent variable was lipid profile according to the fasting and prandial state. The independent variables were sociodemographic, anthropometric parameters and type of food consumed.

Data collection

After the administration of questionnaire and measurement of anthropometric parameters, a first venous blood sample was collected into a dry tube in fasting for at least 12 h. On the next day, subjects were admitted to Parakou Teaching Hospital. Following an overnight fast of at least 12 h, subjects were provided with a food of their choice. Venous blood was sampled at 2, 4 and 6 h following the food intake. The types of foods consumed include:

-Type 1 food: rice + side dish (fry, tomato sauce, cheese, egg);
-Type 2 food: flour paste (maize, dried cassava chips) + sauce (tomato or vegetable) or pounded yam + sauce as side dish (tomato, groundnut, vegetable or gluey sauce) or *akassa* (that is a cooked, ground and soaked maize paste) + side dish (onion, tomato pepper juice);
-Type 3 food: porridge (maize, millet) + wheat doughnuts or fritters;
-Type 4 food: alimentary paste (spaghetti, couscous) or bread + sandwich; biscuits; cake;
-Type 5 food: beans + side dish (groundnut oil or palm oil, cassava flour).

The blood samples (4 mL) collected were centrifuged at 1500 g for

Table 1. General characteristics of study target population.

Parameter	Mean values±SD			
	Total	Male (n=58)	Female (n=42)	P#
Age (years)	31.21±12.36	32.31±12.34	29.69±12.39	*0.298*
BMI (kg/m^2)	23.97±4.56	23.18±3.95	25.07±5.13	0.040
SBP (mmHg)	116.61±21.86	119.33±25.44	112.86±15.14	0.145
DBP (mmHg)	75.06±14.67	74.22±16.24	76.21±12.29	0.506

N=100. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; SD: standard deviation; #: Student's-t independent.

Table 2. Distribution of mean values of fasting serum lipid parameters (in g/L) according to subjects' sex.

Parameter	Total	Male (n=58)	Female (n=42)	P#
Total cholesterol	1.49±0.35	1.44±0.35	1.55±0.36	0.137
HDL cholesterol	0.50±0.15	0.48±0.16	0.53±0.12	0.082
LDL cholesterol	0.85±0.37	0.82±0.38	0.89±0.35	0.322
Triglycerides	0.70±0.35	0.74±0.39	0.64±0.27	0.176

N=100. #Student's-t independent.

5 min, and then serums were decanted. The latter were used on the same day to measure the lipid parameters.

Total cholesterol was measured through endpoint enzyme assay with cholesterol oxidase (MacLachlan et al., 2000), HDL cholesterol measured using phosphotungstic acid-magnesium precipitation procedure (Warnick et al., 1979), and triglycerides measured using endpoint enzyme assay with glycerophosphate oxidase (Solera, 2000). LDL cholesterol was calculated with the formula of Friedewald et al. (1972) under the conditions of triglyceridemia lower than 3.5 g/L.

Data analysis

Statistical analyses were performed with the SPSS software (2011, IBM Corporation). Analysis of variance (ANOVA) and Student's t independent test were used to determine if differences in fasting and non-fasting measurements for total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides were statistically significant. Chi-square independence test of Pearson was used to determine the association between type of food and dyslipidemia. Significance level was set at 0.05.

Ethical considerations

The research protocol of this study was approved by the Local Ethics Committee for Biomedical Research of the University of Parakou (Opinion No. 0302/CLERB-UP/P/SP/R/SA).

RESULTS

General characteristics of study target population

The number of participant was 100, and 58% of them were men (sex-ratio = 1.38). Their mean age was 31.21±12.36 years and mean body mass index (BMI) was 23.97±4.56kg/m2. Mean systolic blood pressure was 116.61±21.86 mmHg and their mean diastolic blood pressure was 75.06±14.67 mmHg (Table 1). Women had a significantly higher body mass index (P=0.040).

Fasting serum lipid parameters

Mean values of fating serum lipid parameters were presented in Table 2. There was no significant difference of fasting serum lipid parameters according to the sex (P>0.05).

Postprandial lipid parameters' values

Table 3 shows the comparison of fasting and postprandial serum lipid parameters among male subjects. The levels of triglycerides after meal were increased compared to fasting triglycerides values, and their changes were statistically significant at 2 h (P=0.000) and 4 h (P=0.004), when analyzed using student's t- independent test. The highest levels of triglycerides were noted 2 h after meal (1.12±0.58 g/L). There was no significant difference between fasting and non-fasting serum lipid parameters among female subjects (P>0.05) when analyzed using both student's t- independent and ANOVA test (Table 4).

Table 5 shows the comparison of fasting and postprandial serum lipid parameters among all the 100 subjects. Except for triglycerides, there were no substantial changes in the distributions of lipid parameters as a function of time since meal intake.

Table 3. Comparison of mean values of fasting and postprandial serum lipid parameters (in g/L) among male subjects.

Parameter	T0 MV±SD	T2 MV±SD	T4 MV±SD	T6 MV±SD	P*	$P(T_0;T_2)^{\#}$	$P(T_0;T_4)^{\#}$	$P(T_0;T_6)^{\#}$
TC	1.44±0.35	1.52±0.46	1.51±0.49	1.51±0.47	*0.774*	*0.322*	*0.382*	*0.375*
HDLC	0.48±0.16	0.44±0.16	0.44±0.14	0.45±0.15	*0.532*	*0.230*	*0.205*	*0.311*
TG	0.74±0.39	1.12±0.58	0.99±0.51	0.86±0.48	*0.000*	*0.000*	*0.004*	*0.122*
LDLC	0.82±0.38	0.84±0.37	0.88±0.42	0.89±0.40	*0.736*	*0.768*	*0.432*	*0.315*

N=58. TC: Total cholesterolemia; HDLC: HDL cholesterolemia; LDLC: LDL cholesterolemia; TG: Triglyceridemia; MV±SD: Mean value±Standard deviation; $P(T_0;T_2)$: P-value of comparison of the mean values of fasting serum lipid parameters and two hours after meal; $P(T_0;T4)$: P-value of comparison of the mean values of fasting serum lipid parameters and four hours after meal; $P(T_0;T6)$: P-value of comparison of the mean values of fasting serum lipid parameters and six hours after meal.
*: ANOVA test; $^{\#}$: Student's-t independent.

Table 4. Comparison of fasting and postprandial serum lipid parameters (in g/L) among female subjects.

Parameter	T0 MV±SD	T2 MV±SD	T4 MV±SD	T6 MV±SD	P*	$P(T_0;T_2)^{\#}$	$P(T_0;T_4)^{\#}$	$P(T_0;T_6)^{\#}$
TC	1.55±0.36	1.54±0.32	1.51±0.37	1.52±0.38	*0.957*	*0.878*	*0.617*	*0.709*
HDLC	0.53±0.12	0.51±0.10	0.51±0.11	0.50±0.11	*0.608*	*0.543*	*0.400*	*0.205*
TG	0.64±0.27	0.79±0.43	0.75±0.42	0.67±0.35	*0.229*	*0.067*	*0.144*	*0.715*
LDLC	0.89±0.35	0.87±0.29	0.85±0.34	0.89±0.36	*0.932*	*0.717*	*0.582*	*0.971*

N=42. TC: Total cholesterolemia; HDLC: HDL cholesterolemia; LDLC: LDL cholesterolemia; TG: Triglyceridemia; MV±SD: Mean value±Standard deviation; P(T0;T2): P-value of comparison of the mean values of fasting serum lipid parameters and two hours after meal; $P(T_0;T4)$: P-value of comparison of the mean values of fasting serum lipid parameters and four hours after meal; $P(T_0;T6)$: P-value of comparison of the mean values of fasting serum lipid parameters and six hours after meal. *: ANOVA test; $^{\#}$: Student's-t independent.

Table 5. Comparison of mean values of fasting and postprandial serum lipid parameters (in g/L) among all study subjects.

Parameter	T0 MV±SD	T2 MV±SD	T4 MV±SD	T6 MV±SD	P*	$P(T_0;T_2)^{\#}$	$P(T_0;T_4)^{\#}$	$P(T_0;T_6)^{\#}$
TC	1.49±0.35	1.53±0.41	1.51±0.44	1.51±0,43	*0.925*	*0.472*	*0.681*	*0.629*
HDLC	0.50±0.15	0.47±0.14	0.47±0.13	0.47±0,13	*0.352*	*0.187*	*0.135*	*0.129*
TG	0.70±0.35	0.98±0.54	0.89±0.49	0.78±0,44	*0.000*	*0.000*	*0.001*	*0.135*
LDLC	0.85±0.37	0.85±0.34	0.87±0.38	0.89±0,38	*0,847*	*0.981*	*0.762*	*0.439*

N=100. TC: Total cholesterolemia; HDLC: HDL cholesterolemia; LDLC: LDL cholesterolemia; TG: Triglyceridemia; MV±SD: Mean value±Standard deviation; $P(T_0;T_2)$: P-value of comparison of the mean values of fasting serum lipid parameters and two hours after meal; $P(T_0;T4)$: P-value of comparison of the mean values of fasting serum lipid parameters and four hours after meal; $P(T_0;T6)$: P-value of comparison of the mean values of fasting serum lipid parameters and six hours after meal.
*: ANOVA test; $^{\#}$: Student's-t independent.

Triglycerides increased after meal among all subjects of the study; the highest levels of triglycerides were noted 2 h after meal (0.98±0.54). Triglyceridemia was significantly higher 2 h (0.70±0.35 g/L vs 0.98±0.54 g/L; P=0.000; 40% increased) and 4 h (0.70±0.35 g/L vs 0.89±0.49 g/L; P=0.001; 27% increased) in postprandial state when analyzed using student's t- independent test. ANOVA test showed significant difference between mean values of triglyceridemia in fasting (0.70±0.35 g/L), 2 h (0.98±0.54 g/L), 4 h (0.89±0.49 g/L) and 6 h (0.78±0,44 g/L) after meal (P=0.000).

The association between types of food and dyslipidemias 6 h after meal was presented in Table 6.

Six hours after meal, type 1 food (rice + side dish (fry, tomato sauce, cheese, egg)) was associated with mixed hyperlipidemia (P = 0,022) when analyzed using chi-

Table 6. Distribution of dyslipidemias according to foods consumed 6 h after meal.

Type of food	Dyslipidemias six hours after meal						
	Total N=100	THC N=14	HDLH N=32	LDLH N=14	HTG N=9	MHL N=5	AD N=4
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Type 1 food							
Yes	20(20.00)	3(21.43)	6(18.75)	2(14.29)	3(33.33)	3(60.00)	0(0.00)
No	80(80.00)	11(78.57)	26(81.25)	12(85.71)	6(66.67)	2(40.00)	4(100.00)
$P^{§}$	-	0.885	0.830	0.564	0.295	0.022	0.307
Type 2 food							
Yes	18(18.00)	2(14.29)	8(25.00)	2(14.29)	2(22.22)	1(20.00)	2(50.00)
No	82(82.00)	12(85.71)	24(75.00)	12(85.71)	7(77.78)	4(80.00)	2(50.00)
$P^{§}$	-	*0.696*	*0.211*	*0.696*	*0.730*	*0.905*	*0.089*
Type 3 food							
Yes	23(23.00)	3(21.43)	7(21.88)	5(35.71)	1(11.11)	0(0.00)	1(25.00)
No	77(77.00)	11(78.57)	25(78.13)	9(64.29)	8(88.89)	5(100.00)	3(75.00)
$P^{§}$	-	*0.880*	*0.854*	*0.223*	*0.374*	*0.210*	*0.923*
Type 4 food							
Yes	23(23.00)	5(35.71)	8(25.00)	5(35.71)	1(11.11)	1(20.00)	0(0.00)
No	77(77.00)	9(64.29)	24(75.00)	9(64.29)	8(88.89)	4(80.00)	4(100.00)
$P^{§}$	-	*0.223*	*0.744*	*0.223*	*0.374*	*0.870*	0.265
Type 5 food							
Yes	6(6.00)	1(7.14)	2(6.25)	1(7.14)	0(0.00)	0(0.00)	0(0.00)
No	94(94.00)	13(92.86)	30(93.75)	13(92.86)	9(100.00)	5(100.00)	4(100.00)
$P^{§}$	-	*0.846*	*0.942*	*0.846*	*0.427*	*0.562*	*0.606*

THC: Total hypercholesterolemia; HDLH: HDL hypocholesterolemia; LDLH: LDL hypercholesterolemia; HTG: Hypertriglyceridemia; MHL: Mixed hyperlipidemia; AD: Atherogenic dyslipidemia; §: Chi-square test of Pearson.

square test of Pearson.

DISCUSSION

The results of this study showed that there was no significant difference between the values of total cholesterol, HDL cholesterol and LDL cholesterol (P>0.05) when compared fasting and postprandial lipid parameters. However, there would be a significant difference between fasting and non-fasting triglyceridemia at 2 h (P=0.000) and 4 h (P=0.001).

Other recent research works showed the same results. In the study of Feres et al. (2018), meal did not influence total cholesterol, HDL cholesterol and LDL cholesterol, but triglycerides increased significantly after meal : 2 h (156.0±86.4 mg/dL, P=0.000), 3 h (148.5±92.0 mg/dL, P=0.000) and 4 h (143.4±93.0 mg/dL, P= 0.000). Among hemodialysis patients, post-prandial triglycerides was significantly raised both in male subjects (1.47±0.99 versus 1.67±1.22 mmol/L, P=0.015) and females (1.56±0.08 versus 1.83±0.11 mmol/L, P=0.001) (Alsaran et al., 2009). An Indian study conducted from 2012 to 2014 concluded that triglycerides were significantly increased till 6 to 7 h after meal (Gupta et al., 2016).

Another study mentioned a significant difference in plasma triglyceridemia which has already increased one hour after the last meal and remained high until 7 h later (Nordestgaard et al., 2009). A considerable difference between fasting and non-fasting triglycerides was noted by Pati and Singh (2017). Rahman et al. (2018) observed a non-fasting triglycerides increased by approximately 20%.

In contrast to above-mentioned studies**,** in a survey conducted among subjects with type 2 diabetes who consumed a standardized meal, Lund et al. (2011) highlighted that total cholesterol and HDL cholesterol mean concentrations have each decreased by approximately 0.1 mmol/L in the postprandial state, whereas triglycerides' mean concentrations rose by 0.8

mmol/L; on the contrary, LDL cholesterol considerably declined (P=0.005).

Postprandial increase in triglycerides may be due to their metabolism. In the postprandial state, when metabolic demands are met, the excess energy resulting from foods is directed towards the synthesis of triglycerides and lipogenesis. In the fasted state, the lipids stored as triglycerides might be used to provide energy through the β-oxidation of fatty acids**,** and to ensure gluconeogenesis (Harvey and Ferrier, 2011). According to Kolovou et al. (2019), overproduction and decreased catabolism of triglycerides-rich remnant lipoproteins are the two main mechanisms leading to postprandial lipid and lipoprotein abnormalities. Triglycerides-rich remnant lipoproteins are a spectrum of particles, some of which are almost as small as LDL particles although most remnant particles are larger and have a lower potential to traverse endothelial cells than the smaller LDL particle. On the other hand, triglycerides-rich remnant lipoproteins are 5-20 times richer in cholesterol content compared with an LDL particle and because of the larger size of triglycerides-rich remnant lipoproteins compared with the smaller size of LDL particles, once they have penetrated into the intima, they may remain there for longer. Other factors influencing lipid profile results may explain increased triglycerides in the postprandial state: meals containing fats, alcohol consumption during or before meal, and other macronutrients (Bae et al., 2003; Mora et al., 2008).

Six hours after meal, only type 1 food was associated with mixed hyperlipidemia (P=0,022). The composition of the type 1 food containing the accompanying sauce, which is richer in fats than the 4 other foods, and eggs could raise cholesterol and triglycerides found in mixed hyperlipidemia; however this observation remains to be confirmed. Lipid concentrations vary widely after meals depending on the postprandial time and the type of food intake. Individual responses to food ingestion are extremely heterogeneous. In addition, there are cultural differences to food ingestion. Standardization is key to establishing a robust laboratory test. For each non-fasting condition, a clinical laboratory should have reference values established for several factors other than the lipid profile (Lund and Jensen, 2011).

Standardizing the procedures and the patient preparation for a fasting protocol is hard enough, and the problem becomes even more complex with a non-fasting protocol (Lorenzo Lozano et al., 2017). A recent American Heart Association statement on hypertriglyceridemia and coronary heart disease suggests that clinicians can use a non-fasting triglycerides level of >200 mg/dL to identify hypertriglyceridemic states (Miller et al., 2011). Among normotriglyceridemic subjects (that is, fasting triglycerides levels <150 mg/dL), consumption of a low-fat breakfast (typically <15 g of fat) before blood sampling should not induce an increase in postprandial triglycerides levels by more than 20%, and is unlikely to cause levels to exceed 200 mg/dL (Dubois et al., 1998). Additional more recent data have suggested that a nonfasting triglycerides level of 175 mg/dL could also be reasonable (White et al., 2015). Follow-up fasting triglycerides testing in these cases are not needed, but this should not dissuade further discussion of lifestyle measures.

The authors hypothesized that there was no difference in fasting and non-fating serum lipid parameters in adult subjects. Our findings show that levels of lipids parameters after normal food intake differ only minimally from levels in the fasting state. It may therefore be concluded that consumed foods do not cause most dyslipidemias during 6 hours in the postprandial state. As in studies conducted by Nordestgaard (2017), Mora et al. (2019) and Yang et al. (2020), the fasting state is not necessary for the screening of dyslipidemias among Benin adult subjects, given the wide variety of foods consumed. From above findings we can affirm that non-fasting blood draws may be highly effective and practical for lipid profile testing.

Conclusion

This research work, focused on variations in serum lipid parameters depending on the prandial state among adult subjects, points out that total cholesterolemia, HDL cholesterolemia and LDL cholesterolemia do not significantly vary whatever the prandial state is. However triglyceridemia significantly raised until four hour in the postprandial state with return to normal six hour later. Only food rich in fats consumed is associated with mixed hyperlipidemia, six hours after meal. It is recommended that non-fasting blood samples be routinely used for the assessment of plasma lipid profiles after a 6-h fasting among adult subjects.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Evaluation of phytochemical constituents, proximate contents and glycemic index of bambara groundnut *(Vigna subterranea* L. Verdc) varieties grown in Northeastern Nigeria

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Bambara groundnut (*Vigna subterranea* L. Verdc) is widely used as a source of food and also has rich fibre and nutritional values. Hence, this research aimed at determining the phytochemical, proximate content as well as glycemic index and glycemic load of four varieties of Bambara groundnut found in Alkalere area of Bauchi State northeastern Nigeria for possible nutritional and medicinal utilization. Seeds of Bambara groundnut varieties were processed by heating at 60°C and cooled then grinded into powder using a mechanical grinder. The flour was used for phytochemical and proximate analysis as well as glycemic index (GI) and glycemic load (GL) determinations. The results revealed the presence of saponins, tannins, steroids, cardiac glycosides, alkaloids and flavonoids in all the varieties while anthraquinones was not detected in all the four varieties. Proximate analysis showed high contents of carbohydrates and protein with low ash content in all the four varieties. The study also found that black seeds of Bambara groundnut had the lowest GI of 66.1 and GL at 1.33 while Brown-black seeds Bambara groundnut was the highest values in GI 75.6 and GL 1.51, respectively. The study for the first time reported the GI and GL differed among varieties with different seed color of different varieties of Bambara groundnut where a black seed variety of Bambara groundnuts possessed some good qualities for use in the nutritional management of blood glucose likely for diabetic patients.

Key words: Bambara groundnut, phytochemical properties, proximate, glycemic index, glycemic load.

INTRODUCTION

Bambara nut (*Vigna subterranea* L. Verdc or *Voandzeia subterranea)* is one of the food legumes. It is an indigenous leguminous African crop that is grown across the continent (Olanipekun et al., 2012). It is commonly found in Nigeria and known locally as; "*Okpa*" (Igbo), "*Epa-roro*" (Yoruba) and "*Kwaruru*" or "*Gurjiya*" (Hausa). There are seven varieties of Bambara groundnut which is mainly recognized by their seed-colour or design, including black, red, cream/black eye, cream/brown eye, cream/no eye, speckled/flecked/spotted purple and

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brown (light or dark) (PGBG, 2011). The traditional use of Bambara groundnut seeds in treatment/management of several ailments is remarkable and presents a gap for detailed study on the therapeutic and pharmaceutical value of the crop (Harris et al., 2018). Jideani and Diederick (2014) reported that the medicinal role of Bambara groundnut is mainly based on information obtained from communities in several parts of Africa and the world where this crop is reportedly responsible and useful for treatment of various ailments. For example as a treatment/management for diarrhea, a mixture of Bambara groundnut seeds and water from boiled maize are consumed. To alleviate the nausea associated with pregnancy, pregnant women chew and swallow Bambara groundnut seeds (Olanipekun et al., 2019). Other prophylactic and therapeutic use of Bambara groundnut seeds includes protein deficiency kwashiorkor, veneral diseases, polymenorrhea (roasted BGN seeds are used), internal bruising, and cataracts (mixture of water and crushed BGN seeds are used (Olanipekun et al., 2019).

Glycemic index (GI) is referring to a measure of the glycemic effect of carbohydrate in a particular food, which is compared to an equivalent amount of carbohydrate in a standard amount of glucose (Jenkins et al., 1981). Studies showed that dietary GI, or GL plays a significant role in glycemic control of an individual (Thomas and Elliott, 2009). Carbohydrate foods with low GI has been considered to be advantageous for its sustained blood glucose levels and metabolic control as reported by Eleazu (2016) where glycemic index of less than 55 is considered low, 56-69 is considered medium and greater than 70 is high. According to Russell et al. (2016), the understanding of the effect of food on glycemic regulation and on the underlying metabolic derangements plays a vital role in the nutritional management of blood glucose levels for the prevention and management of diseases like diabetes mellitus. One of the objective of this study was to ascertain the GI of seed of Bambara groundnut varieties on healthy rats for possible utilization in management of diabetes.

MATERIALS AND METHODS

Plant sample

The four varieties of Bambara groundnut *(Vigna subterranea* L Verdc.) seeds were purchased directly from farmers in Alkaleri LGA of Bauchi State, North-East Nigeria. They were identified by a taxonomist at the Department of Biological Sciences, Abubakar Tafawa Balewa University, Bauchi, Nigeria. They were given a voucher number (Brown-black/ALK/01, Red/ALK/02, Brown/ ALK/03, and Black/ALK04).

Feed formulations

The formulated feed containing seed flour of Bambara groundnut varieties composed seed flour (56%), cray-fish (20%), vegetable oil (5%), rice bran (4%), sucrose (10%), and vitamin/mineral mixture (5%), respectively while the basal feed composed of the same ingredients as the experimental feeds except for Bambara groundnut seed flour was replaced with corn-flour (56%) as described by Olubunmi et al. (2017). The Bambara groundnut seed varieties formulated feeds containing seed flour of Bambara groundnut were tagged as, ALK/01, ALK/02, ALK/03 and ALK/04 feed.

Phytochemical screening

Phytochemical tests were carried out by using the standard methods of Harborne (1999), Sofowora (1993), Trease and Evans (1978) and Savithramma et al. (2011).

Analysis of proximate contents of *Balanites aegyptiaca* kernels

Ten grams of the flour were soaked in 100 ml of pre-boiled distilled water. The solution was shaken vigorously and allowed to stand for 24 h. It was then filtered using Whatman's No. 1 filter paper and concentrated by freeze-drying to solvent free extract. The moisture, ash, fibre carbohydrate, crude protein and fat contents in the seed extract were determined as described by AOAC standard assay method (AOAC, 1997).

Determination of GI and GL

Glycemic index in seeds of four Bambara groundnut varieties was determined in rats as done by Eggum et al. (1982). Briefly, rats were fasted overnight for 12 h and their fasting blood glucose were measured from their tail vein using On Call-Plus Glucometer. Different rats consumed the test diets (Bambara groundnut seed formulated feeds) containing 2 g of carbohydrate for 15 min, while control rats received glucose (2 g/2 ml) solution. Blood glucose levels were again checked at 30, 60, 90 and 120 min. GI was determined by calculating incremental area under 2 h of blood glucose response or curve (IAUC) for each diet and divided with the IAUC for glucose solution (standard) times 100 as reported by Jenkins et al. (1981). GL was calculated from GI value time's for available carbohydrate in diet divided by 100 (Wolever et al., 2003).

RESULTS

The phytochemical contents of 4 varieties of Bambara groundnut (Brown-black, Red, Brown and black) are shown in Table 1, saponins, tannins, steroids, cardiac glycosides, alkaloids and flavonoids were detected in all the varieties while anthraquinones were not detected in all the 4 varieties.

Proximate composition of different varieties of Bambara groundnut is shown in Figure 1. The results showed all the seed varieties are rich in carbohydrate and protein contents but low in crude fat, moisture and ash. The red and black seeds are highest in carbohydrate content whereas, brown-black and brown seeds are highest in protein content. All the seeds varieties showed to contain low crude fat, moisture and ash where red and brown seeds contained the lowest crude fat, brown-black and brown seeds are lowest in moisture and, red and black seeds lowest in ash content. The low ash content indicates their low mineral contents, while low moisture content shows their ability to be stored for a long period

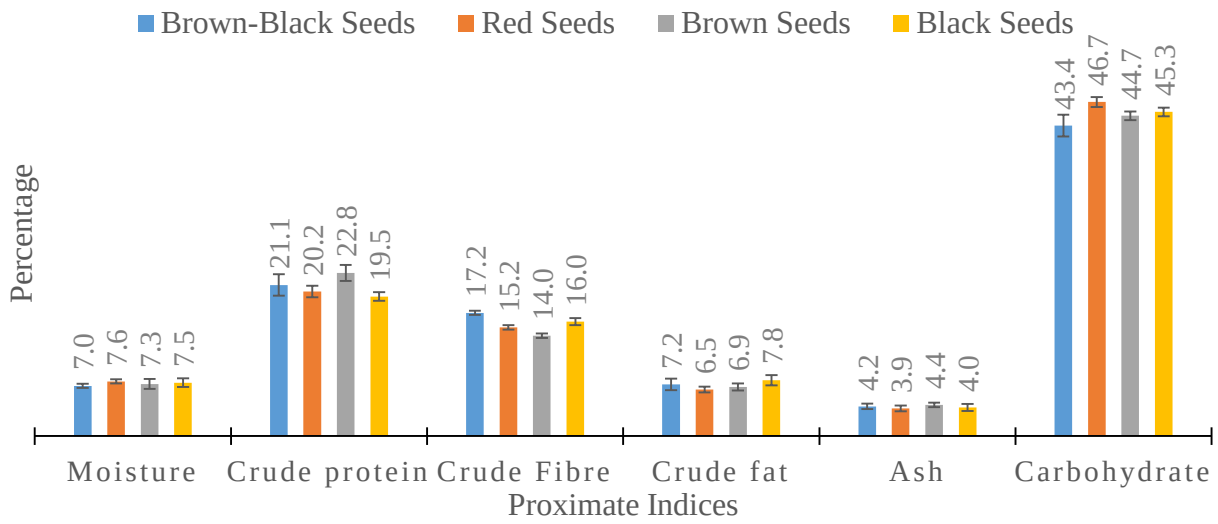

Figure 1. Proximate composition of different varieties of Bambara groundnut as mg/100 g of dry sample.

Table 1. Phytochemical composition in seeds of 4 Bambara groundnut varieties.

Phytochemicals	Brown-black seeds	Red seeds	Brown seeds	Black seeds
Saponins	+	+	+	+
Tannins	+	+	+	+
Steroids	+	+	+	+
Cardiac glycosides	+	+	+	+
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Anthraquione	-	-	-	-

+ Detected, - Not Detected

of time under ambient conditions.

The results of blood glucose response of rats fed using different varieties of Bambara groundnut formulated feeds is presented in Figure 2. The results showed a difference in the increase in blood glucose responses of the tested rats while rat received glucose as standard had blood glucose response raised to 8.6 mmol/L. In the other hand, when rats were fed formulated feed using different seeds of Bambara groundnut varieties, their blood glucose response varied in the range of 4.8-6.7 mmol/L after feeding for 30 min. Sixty min later, the blood glucose responses begun to fall which continued throughout the 90 min duration where rat received glucose had 5.5 mmol/L and those that fed formulated feed using different seeds of Bambara groundnut varieties recorded a 3.5-5.5mmol/L reduction respectively.

The glucose incremental area under the curve (iAUC) values after consumption of the different seed diets of Bambara groundnut varieties varied in a range of 133.5-152.05 mmol/L*120 min as against 201.15 mmol/L*120 min of the standard (glucose) as presented in Figure 3. The study found that black seeds the Bambara groundnut variety had the lowest iAUC value (133.5) as well as low GI (66.1) and GL (1.33) while brown-black seeds of Bambara groundnut variety is the highest in iAUC, GI (75.6), and GL (1.51) respectively as can be seen in Table 2.

DISCUSSION

Plant parts produce different chemical compounds or phytochemicals which have been used in a wide range of commercial, medicinal and industrial applications. The obtained results obtained from the preliminary qualitative phytochemical screening of different Bambara groundnut varieties showed that the seeds of all varieties had almost all the phytochemicals screened. The findings are similar to research of Alhassan et al. (2018) which showed most of phytochemicals existing in *Balanites aegyptiaca* kernels screened. Secondary plant metabolites in plants known as phytochemicals are

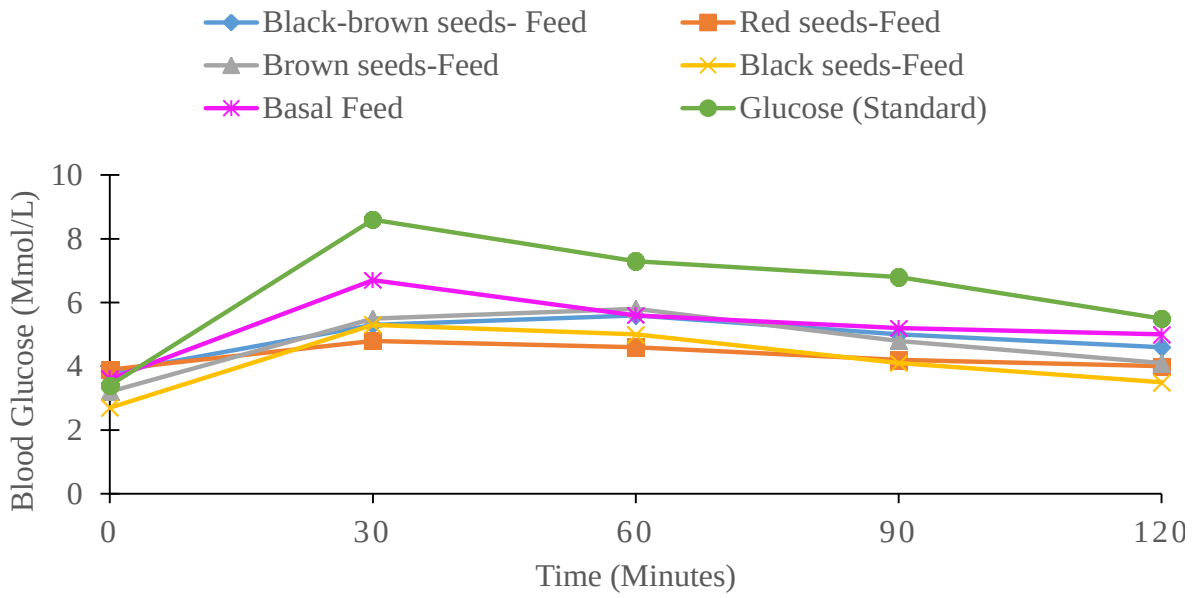

Figure 2. Change in Blood glucose of normoglycemic rats after feeding with different varieties of Bambara groundnut.

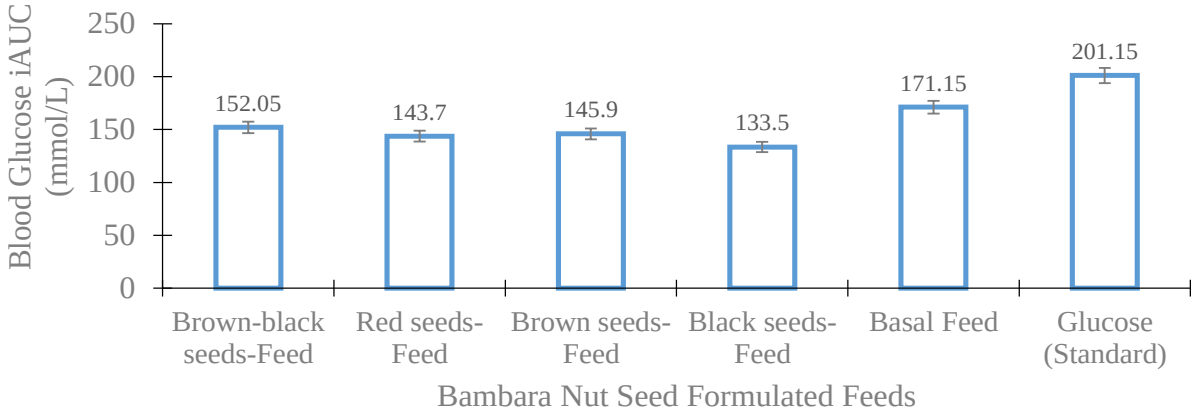

Figure 3. Differences in glucose incremental area under the curve following blood glucose response of rats fed with seed formulated feeds of different Bambara groundnut.

Table 2. GI and GL of different varieties of Bambara groundnut formulated feed following feeding of healthy rats.

Variable	Available carbohydrate (g)	Serving size (g)	Glycemic index	Glycemic load
Brown-black-seeds Feed	2	20	75.6	1.51
Red seeds-Feed	2	20	71.4	1.43
Brown seeds-Feed	2	20	72.5	1.45
Black seed-Feed	2	20	66.4	1.33
Basal-Feed	2	3.5	85.1	1.70
Glucose (Standard)	2	2	100	2.00

known to be important for both plants and animals but could also be harmful or show some adverse effects on animals especially when consumed in large quantities hence called anti-nutrients. Antinutritional factors are known to affect the availability of nutrients required by the body and interfere with metabolic process so that growth

and development of the body is negatively influenced. These anti-nutritional factors can be reduced easily to tolerable limits by proper processing techniques such as soaking, cooking and frying (Mohammed et al., 2019).

Proximate composition of different varieties of Bambara groundnut showed high percent of carbohydrates in all the varieties with red seeds Bambara groundnuts having the highest percent in carbohydrates (46.65%), but low percent of ash (3.85%). The proximate composition of the different varieties of Bambara groundnuts recorded in this study are in-line with the findings of Abdulrahaman et al. (2012) and also similar to what was obtain by Alhassan et al. (2015) on Bambara groundnut grown in Madobi LGA of Kano State-Nigeria, which showed high percent of carbohydrate and low percent of ash.

Furthermore, in this study, it is firstly reported that the GI values differed among Bambara groundnut varieties. The study showed that dietary GI, or GL played a significant role in glycemic control of an individual (Thomas and Elliott, 2009). Where carbohydrate foods with low GI are considered to be advantageous to sustained blood glucose levels and metabolic control. In the present study, GI of different varieties of Bambara groundnut was investigated on healthy rats where different GI values were recorded. Previous research showed that GI of food was influenced by the type and amount of dietary fiber content (Russell et al., 2016). Different GI of the Bambara groundnut varieties recorded in this study could be as a result of their different fiber content as reported by Russell et al. (2016) as well as differences in their physical and chemical characteristic as observed by Foster-Powell et al. (2002).

Literature survey reveals that GI of less than 55 is considered low, 56-69 is considered medium and greater than 70 is high as reported by Kaviani et al. (2020). According to the category the GI of most of the Bambara groundnut varieties falls within the category considered as high with values between 71.4 and 75.6 where only one which is identified as black seed variety of Bambara groundnut has value (66.4) that is considered as medium. The GL of Bambara groundnut varieties determined in the present study provided us with clear picture of how they affect the blood glucose. It was reported that, GL between 1 and 10 is considered low, 11-19 is moderate and 20 or higher is considered as high. Based on this, the GL of all the Bambara groundnut varieties studied are within the criteria considered low hence, the lesser they elevate blood glucose and insulin responses. It was reported that consumption of a diet with high GL is associated with an increased risk of type 2 diabetes and other related diseases (Liu et al., 2000).

Conclusion

The study for the first time reported the GI and GL of different varieties of Bambara groundnut where black seed variety of Bambara groundnut possessed some good qualities that could be consider for use in the nutritional management of blood glucose likely for diabetic patients.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Phytochemical and biological investigations of extracts from the roots of *Cocos nucifera* L. (Arecaceae) and *Carica papaya* L. (Caricaceae), two plants used in traditional medicine

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***Cocos nucifera* L. (Arecaceae) and *Carica papaya* L. (Caricaceae) are two plants used to treat several human pathologies such as oxidative stress and inflammatory diseases. The aim of this study is to assess anti-inflammatory activity, antioxidant activity and acute oral toxicity, of aqueous and methanolic extracts from *C. nucifera* L. and *C. papaya* L. using appropriate experimental models. The acute oral toxicity test of extracts on mice was evaluated using Economic Cooperation and Development (OECD) guidelines 423. The capacity of extracts to inhibit key inflammation enzymes such as 15-lipoxygenase, phospholipase A_2, cyclooxygenases 1 and 2 was evaluated *in vitro*. The antioxidant activity was assessed *in vitro* using four methods (inhibition of lipid peroxidation, FRAP, DPPH and ABTS tests). The phytochemical screening showed the presence of sterols and triterpenes, saponins, flavonoids, phenolics and tannins. Methanolic extract from *C. nucifera* exhibited higher values of 855.06 ± 1.71 mg GAE/ g, 418.22 ± 1.92 mg CE / g, 19.31 ± 0.6 mg GAE / g, 20.6 ± 0.36 mg QE / g dry extract respectively for phenolics, condensed tannins, hydrolysable tannins and flavonoid content. The extracts demonstrated antioxidant capacity, potential to inhibit pro-inflammatory enzymes and a lethal dose (LD_{50}) was estimated to 5000 mg/kg b.w. The results of this study constitute a solid scientific basis that can justify the traditional uses of these plants.**

Key words: *Cocos nucifera, Carica papaya,* phytochemistry, acute oral toxicity, antioxidant, anti-inflammatory.

INTRODUCTION

Herbal medicine has been around since human civilization and continues to play a vital role in health care (Deepika and Yash, 2013). Last decade, several molecules used as drugs were isolated from natural resources on the base of traditional uses (Murugan and Mohan, 2011). Majority of resources are made up of

medicinal plants which possess compounds (secondary metabolites). These compounds are diverse and are responsible several pharmacological properties including antioxidant, anti-inflammatory, antibacterial, antidiabetic, hepatoprotective, etc (Wadood et al., 2013).

Inflammation is the living organism's response to any aggression (pathogens, injury). During inflammation process, inflammatory mediators such as prostaglandins, leukotrienes, cytokines (TNFα, IL1β, IL6) and reactive oxygen species (ROS) are released by cells activation. Prostaglandins and leukotrienes are respectively produced by cyclooxygenases (COX) and lipoxygenase (LOX) activation (Hunter, 2012). Inflammatory mediators and redox status participate in a significant disease process in both acute and chronic inflammatory states. This involves major cascades of release of inflammatory mediators, which are generally associated with oxidative damage to cellular constituents (Sharma et al., 2019).

Oxidative stress and ROS are mainly associated with the pathophysiology of major chronic diseases such as inflammation, cancer, atherosclerosis, diabetes, and arthritis (Chikara et al., 2018). Inflammatory disorders are currently treated with steroidal / nonsteroidal anti-inflammatory drugs that induce side effects (gastric, renal, and cardiovascular disorders) (Jordan and White, 2001).

Medicinal plants used to treat inflammation are a natural source of discovery of anti-inflammatory agents with fewer side effects. According to WHO, around 80% of population in developing countries use plants for their primary health care. However, most of medicinal plants are used world-wide without scientific data about their possible toxic effects. World Health Organization has recommended that medicinal plants used to treat human diseases be the subject of further scientific investigation on their side effects (WHO, 2008; Koriem et al., 2019). So, plants should be studied in order to better understand their efficacy, properties and safety (Owolabi et al., 2007; Koriem et al., 2019).

Cocos nucifera L., belonging to Arecaceae family (palm family) known as coconut and *Carica papaya* L. derived from Caricaceae family are two medicinal plants cultivated currently in the hot and humid countries (America, Africa, India, Brazil). All of their constituents have various benefits to the human body through effects on inflammation, nociception, oxidative stress, fever, dysentery, tumor (Maisarah et al., 2014; Lima et al., 2015). The aim of this study was to determine the phytochemical composition and to assess *in vitro* the antioxidant, anti-inflammatory properties and safety of methanolic and aqueous extracts from the roots of *C. nucifera* L. and *C. papaya* L.

MATERIALS AND METHODS

Chemicals

Trolox was purchased from Fluke, France. Iron dichloride, hydrochloric acid, indomethacin, hydrogen peroxide, ABTS [2,2'-azinobis (3-ethyl benzoin-6- sulphonate)], trichloroacetic acid, aluminum trichloride, ammonia, by Prolabo (Paris, France). Folin Ciocalteu reagent, gallic acid, quercetin, ferric chloride, DPPH (2, 2-diphenyl-1-picrylhydrazyl), trichloroacetic acid, hydrochloric acid, potassium persulfate, ascorbic acid, ketamine, potassium hexacyanoferrate, 2-thiobarbituric acid, sodium tetraborate, boric acid, zileuton, linoleic acid, tween 20, and lipoxygenase (type I-B) enzyme were purchased from Sigma® (St Louis, USA). COX-1 and human COX-2, Screening Kit (Item No. 560131) and sPLA2 (Item No. 765001) were manufactured by Cayman Chemical Co. (MI, USA). All solvents used were of analytical grade.

Plant materials and extraction

C. papaya L. (Caricaceae) roots were harvested in September 2018 around Dedougou in the region of the mouhoun loop located 250 km to the capital of Burkina Faso (N 12°46'44.6; W 003°44'91.4). A sample was identified and authenticated at the Plant Biology and Ecology Laboratory of University Joseph KI-ZERBO. The voucher specimen was deposited under number T4316. *C. nucifera* L. (Arecaceae) roots were provided by a tradipractician and were also authenticated at the same laboratory. The roots of two plants were rinsed with running water, and dried under ventilation out of the dust and light; then, they were powdered by Gladiator Est. 1931 Type BN 1 Mach. 40461 1083.

A quantity of 50 g of each powder was macerated in water (500 ml) for 24 h. After filtration, the extracts were centrifuged and lyophilized to obtain dried extract. A methanolic maceration was realized using the same method but after filtration with whatman's filter paper, the extracts were concentrated with rotary vacuum evaporator and kept in an oven until complete evaporation of solvent. The extracts obtained were kept cool for further investigations.

Animals and ethical approval

Female NMRI mice weighing between 20 - 35 g from the animal's house of Institute of Health Sciences Research were used for toxicological assays of extracts. The animals were maintained at laboratory breeding conditions (temperature of 20 - 25 °C, 12 h light/12 h dark cycle, and humidity of 60%). They were fed with standard laboratory pellet (29% protein) and running water. The laboratory experimentation was carried out according to the experimental protocols already validated by the Institute of Health Sciences Research laboratories and meeting the international standards in this field (guidelines established by the European Union on the protection of animals, CCE Conseil 86/609).

Phytochemical screening

The phytochemical screening of extracts from plants was carried out by thin layer chromatography (TLC, 60 F_{254}, 10 x 5 cm, 10 x 20

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cm glass support, Merck) in accordance with literature methods (Ladigina et al., 1983; Santiago and Strobel, 2013). Each dry extract was solubilized in methanol and deposited on the plate for the evolution of the chromatogram. The purpose of this test was to screen large chemical groups like sterols, triterpenes, flavonoids, tannins, alkaloids, coumarins which are secondary metabolites with several pharmaceutical properties.

Specific reagents were used to reveal these groups of compounds: Dragendorff reagent for alkaloids; 5% ethanol $FeCl_3$ reagent for tannins and phenolics; Neu's reagent for flavonoids; Sulfuric vanillin reagent for terpenes and sterols; Anysaldehyde reagent for saponosides; and 5% methanolic KOH reagent for coumarins.

Phytochemical composition

Total phenolics content

The total phenolics of aqueous and methanolic extracts were carried out according to method described by Singleton et al. (1999). To do this, 25 µl of each extract (100 µg/ml) was mixed with 125 µl of Folin Ciocalteu Reagent (FCR 0.2 N). After 5 min at ambient temperature, 100 µl of sodium carbonate solution (75 g/L) was added. The mixtures were incubated during 1 h at room temperature and the absorbances were measured (Spectrophotometer UV, Epoch Biotek Instruments, U.S.A.) at 760 nm against blank. A standard calibration curve was calculated by using Gallic acid. The mixture made in triplicate and the results were expressed in mg of Gallic acid equivalent per g of extract (mg GAE/g).

Tannins content

Condensed tannins

The method used to determine the condensed tannins content is that described by Swain and Hillis (1959). 1 ml of extract (5 mg/ml) was added to 2 ml of vanillin 1% (1 g of vanillin and 100 ml of 70 % sulfuric acid). After 15 min incubation in water bath at 20°C, the absorbance of the mixture was measured (spectrophotometer UV, Shimadzu) at 500 nm. The condensed tannins content T (%) was determined using the following formula:

$$T\ (\%) = 5.2\times10^{-2} \times (A \times V / P)$$

5.2×10^{-2} = constant in equivalence of cyanidin, A = absorbance, V = extract volume and P = extract weight.
The condensed tannins content of the samples was determined in triplicates and the results were converted to mg of cyanidin equivalent (CE) / g dry extract.

Hydrolyzable tannins

The hydrolyzable tannins were performed to Mole and Waterman (1987) method. 1 ml of each extract (5 mg/ml) was mixed to 3.5 ml of the reagent (ferric chloride $FeCl_3$ 10^{-2} M in hydrochloric acid HCl 10^{-3} M). The absorbance of the mixture was measured (spectrophotometer UV, Shimadzu) at 660 nm after 15 s incubation. The hydrolysable tannins content T (%) was determined according to the formula below:

$$T\ (\%) = (A \times PM \times V \times FD) / \varepsilon\ mole \times P$$

A = absorbance, PM = weight of gallic acid (170.12 g/mol), V = volume of extract, FD = dilution factor, ε mole = 2169 (constant in equivalence of gallic acid), P = extract weight

The hydrolysable tannins content of the samples was determined in triplicates and the results were converted to mg of gallic acid equivalent (GAE) / g dry extract.

Flavonoids content

The total flavonoïds of the extract were measured by aluminium chloride ($AlCl_3$) colorimetric assay (Arvouet-Grand et al., 1994). Each extract (1 mg) was dissolved in methanol (1 mL). 100 µL of $AlCl_3$ solution (2 % in methanol) was added to equal volumes of extracts. After shaking, the mixture was incubated for 10 min, and the absorbance was measured at 415 nm with spectrophotometer (Spectrophotometer UV, Epoch Biotek Instruments, U.S.A.) against blank. The blank was composed of 100 µL methanol and 100 µL of each extract. Quercetin was used to produce the standard curve. The flavonoïd content of extracts was determined in triplicates and the results were expressed as mg of quercetin equivalent (QE)/g dry extract.

Anti-inflammatory activity

Phospholipase A_2 inhibition assay

The $sPLA_2$ inhibition test from Cayman Laboratories allows the screening of $sPLA_2$ inhibitors (Type V). The assay was determined with the method described by Cayman Chemical Co. (MI, USA) in the catalog No. 765001. The assay was done in triplicate using 96-wells microplate. The absorbances were read (Agilent 8453) at 405 nm against a blank that had not received the enzyme. Ascorbic acid was used as reference compound and $sPLA_2$ inhibition percentage per 100 µg/mL (final concentration in the wells) was calculated with the formula:

$$\%\ Inhibition = [(AEA - AIA) / AEA] \times 100$$

Where, AEA: Activity enzyme test absorbance; AIA: Activity inhibition test Absorbance

Cyclooxygenases 1 and 2 inhibition assay

The cyclooxygenases Cox-1 (ovine) and Cox-2 (ovine recombinant) inhibition assay was carried out using a commercial colorimetric inhibitor test kit (Catalog No. 560131, Cayman Chemical Company, U.S.A). The tests were carried out following manufacture's instructions. Analysis was performed spectrophotometrically Epoch (Bioteck Instruments, U.S.A) at wavelength of 590 nm against blank. Indomethacin and ascorbic acid were used as reference compound and percentage of inhibition induced by 100 µg/mL was given by the formula:

$$\%\ Inhibition = [(AEA - AIA) / AEA] \times 100$$

Where, AEA: Activity enzyme test absorbance, AIA: Activity inhibition test Absorbance

Lipoxygenase inhibition assay

The inhibition of lipoxygenase was determined by Malterud and Rydland (2000)'s method. For this purpose, 3.75 µl of the extracts at concentration of 8 mg/mL was mixed with 146.25 µl of lipoxygenase solution (820.51 U/ml) prepared in boric acid buffer (pH 9, 0.2 M). The mixture was incubated at ambient temperature during 3 min and 150 µl of 1.25 mM of linoleic acid (substrate) was

added. Spectrophotometer **(**Epoch Biotek Instruments, U.S.A.) was used to record the absorbances for 3 min at 234 nm. The tests were performed in triplicate and zileuton was used as reference compound. The percentage of lipoxygenase inhibition was calculated using the formula:

% Inhibition = $(V_b - Vs) / V_b \times 100$

Vb: Enzymatic activity without inhibitor; Vs Sample: Enzymatic activity with sample/reference compound

Antioxidant activity

Lipid peroxidation inhibitory test (LPO)

The inhibitory capacity of lipid peroxidation activity was evaluated with rat liver using Sombié's method (Sombié et al., 2011). 0.2 ml of extracts or positive control (ascorbic acid) at a concentration of 1.5 mg / ml was mixed with 1 mL of liver homogenate in 10% phosphate buffered saline (PBS) buffer (pH 7.4), 50 µl of $FeCl_2$ (0.5 mM) and then 50 µl of H_2O_2 (0.5 mM). After one-hour incubation at room temperature, 1 ml of trichloroacetic acid (15 %) and 1 ml of 2-thiobarbituric acid (0.67%) were added. The mixture was incubated for 15 min in boiling water and centrifuged (2000 rpm for 10 min). The absorbances were read with a spectrophotometer (Epoch Biotek Instruments, U.S.A.) at 532 nm against control (without extract). All of these measurements were carried out in triplicate. The percentage of inhibition induced by 100 µg/ml was calculated as follows:

%Inhibition = $(A_b - A_e) / A_b \times 100$

A_b: absorbance of control; A_e: absorbance of extracts/ reference compound

FRAP (ferric reducing antioxidant power) test

The ability of extracts to reduce Fe^{3+} ion to Fe^{2+} ion was evaluated using the method described by Hinneburg et al. (2006). To 500 µl of each extract (1 mg/ml), were added 1.25 ml of phosphate buffer (pH 6.6, 0.2 M), and then 1.25 ml of potassium hexacyanoferrate solution $[K_3Fe(CN)_6]$ (1% in water). After 30 min incubation in a water bath at 50°C, 1.25 ml of trichloroacetic acid (10 %) was added and the mixture was centrifuged (2000 rpm) for 10 min. 625 µl of the supernatant was mixed with 625 µl of distilled water and then 125 µl of freshly prepared 0.1% $FeCl_3$ in water. A blank without sample is prepared under the same conditions. The absorbances were read at 700 nm with spectrophotometer (Epoch Biotek Instruments, U.S.A) against a standard curve of ascorbic acid. The potential of extracts to reduce iron (III) to iron (II) was expressed in millimole Ascorbic Acid Equivalent per gram of dry extract (mmol AAE/g).

DPPH (2,2-diphenyl-1-picrylhydrazyl) test

The capacity of extracts to scavenge DPPH radical was assayed as described by Velazquez et al. (2003). A cascade dilution of the extract and reference substances (Trolox and ascorbic acid) was performed from a concentration of 1 mg/ml. For this purpose, 200 µl of 4% DPPH solution (in methanol) freshly prepared was mixed with 100 µl of each dilution in the 96-wells microplate. The mixture was incubated for 30 min at ambient temperature. The absorbances were read (Epoch Biotek Instruments, U.S.A.) at 517 nm against a blank (methanol). The percent inhibition was calculated as follows:

% Inhibition = $(A_b - A_e) / A_b \times 100$

A_b: absorbance of blank; A_e: absorbance of extract/reference compound

ABTS (2, 2'-azinobis- [3-ethylbenzothiazoline-6-sulfonic acid]) test

ABTS radical cation scavenging ability of extracts was determined according to Re et al. (1999)'s procedure. On the eve of the test, a stock solution of ABTS (7 mM) was prepared with 2.45 mM of potassium persulfate ($K_2S_2O_8$) and the mixture was stored in the room without light for 12 to 16 h. A cascade dilution range of the extracts and reference substances (Trolox and ascorbic acid) were realized from a concentration of 1 mg/ml. 20 µl of each dilution was mixed with 200 µL of the ABTS solution diluted in ethanol in the 96-wells microplate. The absorbances were read against blank (ethanol) on a spectrophotometer (Epoch Biotek Instruments, U.S.A.) at 734 nm, after 30 min of incubation in the dark at room temperature. The ABTS radical inhibition was determined by the formula below:

% Inhibition = $[(A_b - A_e) / A_b] \times 100$

A_b: absorbance of blank; A_e: absorbance of extract/reference compound

Acute toxicity test

The acute toxicity study was conducted according to acute toxic class method of the Organization for Economic Cooperation and Development (OECD, 2001) test guideline 423. The test was carried out twice.

Administration of extracts

Before the experiment, all the female mice were weighed, marked, and grouped randomly into five batches with three mice / group. After four hours fasting with access to running water, the control group received distilled water at a dose of 10 ml/ kg, the aqueous and methanolic extracts from *Carica papaya* were administered to the animals of batch 2 and 3 respectively; and the aqueous and methanolic extracts from *Cocos nucifera* were administered to the animals of batch 4 and 5 respectively. All of the extracts were administered to the animals at a single dose of 2000 mg/kg of body weight by oral gavage.

Daily observations

After oral gavage, the animals were observed for 2 h while animals were monitored individually and next, they were fed. All the animals were inspected individually with particular attention for fourteen days in order to detect any sign of toxicity namely general behavioral in eyes, skin, activeness, touch, and movement changes including number of deaths.

Statistical analysis

The data were expressed as Mean ± Standard Error of Mean (SEM). The statistical analysis was carried out according to one-way ANOVA analysis followed by Dunnett's test compared to the control and between methanolic and aqueous extract on Graph Pad Prism software version 6.0. The level of significance was accepted at $p < 0.05$.

Table 1. Phytochemical screening of methanolic and aqueous extracts from *C. nucifera and C. papaya* roots by TLC.

Chemical groups	Extracts			
	CN M	CN A	CP M	CP A
Flavonoids	+	+	+	+
Coumarins	-	-	-	-
Alkaloids	-	-	-	-
Terpenes and sterols	+	+	+	-
Tannins and phenolics	+	+	+	+
Saponins	+	+	+	+

CN M: *C. nucifera* methanolic extract; CN A: *C. nucifera* aqueous extract; CP M: *C. papaya* methanolic extract; CN A: *C. papaya* aqueous extract; -: not detected; +: detected.

Table 2. Total phenolics, condensed tannins, hydrolyzable tannins and total flavonoids contents of methanolic and aqueous extracts from *C. nucifera and C. papaya* roots.

Extracts	Phenolics	Condensed Tannins	Hydrolyzable Tannins	Flavonoids
	mg GAE/g	mg CE/g	mg GAE/g	mg QE/g
CN M	855.06 ± 1.71	418.22 ± 1.92	19.31 ± 0.6	20.6 ± 0.36
CN A	465.17 ± 0.43	359.56 ± 0.83	18.14 ± 0.85	17.9 ± 0.33
CP M	159.8 ± 1.92	52.56 ± 0.3	8.01 ± 0.38	7.2 ± 0.13
CP A	113.38 ± 0.96	4.34 ± 0.03	3.93 ± 0.11	5.39 ± 0.44

Mean values ± standard deviation were presented (n = 3); CN M: *C. nucifera* methanolic extract; CN A: *C. nucifera* aqueous extract; CP M: *C. papaya* methanolic extract; CN A: *C. papaya* aqueous extract; GAE: Gallic acid equivalent; CE: Cyanidin equivalent; QE: quercetin equivalent.

RESULTS AND DISCUSSION

Phytochemical study

Phytochemical screening by TLC of *C. nucifera* and *C. papaya* roots extracts revealed the presence of secondary metabolites like flavonoids, saponins, tannins and phenolics and absence of coumarins and alkaloids (Table 1). *Carica papaya* methanolic extract contains sterols and triterpenes while aqueous extracts do not.

The total phenolics, condensed tannins, hydrolysable tannins and total flavonoids contents of methanolic and aqueous extracts from the plants are presented in Table 2. The methanolic extracts presented the highest total content of phenolics, flavonoids and tannins than water extracts. It has also been reported that the extraction of phenolics from the plant samples are influenced by the nature of solvent (Younus et al., 2019). This could be explained by the efficiency of methanol (a very polar solvent) in the degradation of cell walls which have a non-polar character and cause the release of polyphenols from cells. Also, there is an enzyme that degrades polyphenols in aqueous extracts called polyphenol oxidase which may be responsible for the decrease in the activity of the aqueous extraction. In methanol extracts, this enzyme would be absent (Lapornik et al., 2005; Tiwari *et al.,* 2011). Indeed, the phenolics, flavonoids and tannins were well documented as valuable antioxidants and anti-inflammatory (Sharma et al., 2019).

Antioxidant activity

DPPH radical scavenging, $ABTS^{+}$ radical cation decolorization, ferric ion reduction and lipid peroxidation inhibition in rat liver assays were used to assess the antioxidant activity. Indeed, Reddy et al. (2012) specified that it is necessary to realize more than one antioxidant method to take into account the different antioxidant modes of action. The extracts exhibited antioxidant activity with the best activities recorded by methanolic extracts indicated in Table 3. The methanolic extract from *C. nucifera* demonstrated interesting results in ABTS and DPPH assays were highly comparable to Trolox as reference. The results obtained showed that the extract had a capacity to reduce the ferric ion to ferrous ion. In this test, antioxidant electron donation leads to the neutralization of the free radical (Moualek et al., 2016). The *in vitro* inhibition percentage on lipid peroxidation in rat liver of methanolic extracts from both plants at 100 µg/ml was greater than 50%. However, the percentage of ascorbic acid (94.95 ± 0.94) was better than all of the extracts. In general, *C.nucifera* showed better antioxidant

Table 3. Antioxidant activity of methanolic and aqueous extracts from *C. nucifera and C. papaya* roots.

Extracts	ABTS	DPPH	FRAP	LPO
	IC_{50} (µg/ml)	IC_{50} (µg/ml)	mmol AAE/100 g	(% Inhibition at 100 µg/ml)
CN M	4.79 ± 0.06 ^{ns}	1.4 ± 0.08 ^{ns}	87.71 ± 1.42$^{\#\#\#\#}$	55.99 ± 1.3****
CN A	8.00 ± 0.08 ****	1.4 ± 0.05 ^{ns}	70.26 ± 1.98$^{\#\#\#\#}$	42.61 ± 2.36****
CP M	20.19 ± 1.84****	7.78 ± 0.20****	24.12 ± 1.16$^{\#\#\#\#}$	50.82 ± 1.10****
CP A	66.24 ± 0.41****	146.24 ± 0.32****	3.85 ± 0.17$^{\#\#\#\#}$	49.65 ± 0.74****
Trolox	2.04 ± 0.12	1.74 ± 0.002	nd	nd
AA	0.94 ± 0.08 ^{ns}	1.82 ± 0.02 ^{ns}	nd	94.95 ± 0.94

Values are expressed as mean ± SEM, n = 3 (****) = $p < 0.0001$ indicate significance, ns: no significance $P > 0.05$, extracts vs Trolox for ABTS, DPPH, LPO using one-way ANOVA analysis followed by Dunnett's test; ($^{\#\#\#\#}$) = $P < 0.0001$ methanolic extract vs aqueous extract for FRAP. CN M: *C. nucifera* methanolic extract; CN A: *C. nucifera* aqueous extract, CP M: *C.papaya* methanolic extract; CP A: *C. papaya* aqueous extract, AA: Ascorbic acid, AAE: Ascorbic acid equivalent; nd: not determined.

Table 4. Summary of pro-inflammatory enzymes inhibition values 15-lipoxygenase (15-LOX), cyclooxygenases (COX-1 & COX-2), Phospholipase A_2 ($sPLA_2$).

Extracts	15-LOX	COX-1	COX-2	$sPLA_2$
	IC_{50} (µg/mL)	(% inhibition at 100 µg/mL)		
CN M	8.31 ± 0.73***	27.21 ± 1.66****	39.41 ± 1.36****	24.68 ± 0.08****
CN A	24.57 ± 1.16****	nd	nd	nd
CP M	50.00 ± 0.09****	11,99 ± 0,00****	74.62 ± 1.36****	37.72 ± 0.05****
CP A	87.01 ± 0.8****	nd	nd	nd
Zileuton	2.74 ± 0,02	nd	nd	nd
Ascorbic acid	nd	38.56 ± 0.73*	22.82 ± 0.40****	20.95 ± 0.00
Indomethacin	nd	44.93 ± 0.83	53.94 ± 1.43	nd

Values are expressed as mean ± SEM, n = 3 (*) = $p < 0.05$, (****) = $p < 0.0001$ is considered significant compared to the reference compound. Extracts vs zileuton for 15-LOX, extracts vs Indomethacin for COX-1 and COX-2 and extracts vs Ascorbic acid for PLA_2 according to one-way ANOVA analysis followed by Dunnett's test. CN M: *C. nucifera* methanolic extract; CN A: *C. nucifera* aqueous extract; CP M: *C. papaya* methanolic extract; CP A: *C. papaya* aqueous extract; nd: not determined.

power compared to *C. papaya* for the four methods used with the exception of aqueous extracts in the lipid peroxidation test. Peroxidation of lipids disturbs the integrity of cell membranes and lead to rearrangement of membrane structure (Ozougwu, 2016). Inhibition of extracts against lipid peroxidation suggests that extracts protect cell membrane. It has been reported in the literature that free radical plays a crucial role in the pathogenesis of several diseases including inflammation, pulmonary, cancer, rheumatoid, diabetes, cardiovascular diseases, atherosclerosis, hypertension, ischemia/ reperfusion injury (Valko et al., 2007; Reuter *et al.,* 2010). Antioxidants are substances that prevent various pathologic changes in living cell by protecting oxidation of its major constituents (proteins, lipids, carbohydrates and DNA) (Moualek et al., 2016). Phenolic compounds are considered the most antioxidant metabolites from plants; and these compounds have the ability to give hydrogen or electrons (Koolen et al., 2013; Da Silva Santos et al., 2020).

Inhibitor effect extracts against enzymes

The results of the proinflammatory enzymes inhibition tests are presented in Table 4. These results showed that methanolic extract of *C. nucifera* presented the high inhibition activity of 15-lipoxygenase. However, Zileuton (reference compound) exhibited better IC_{50} in comparison to the extracts. *C. papaya* methanolic extracts were significant highly comparable with Indomethacin and ascorbic acid used as reference compounds, respectively in COX-2 and PLA_2 inhibition. Inflammation is the part of biological reaction of vascular tissues to external harmful stimuli, such as pathogens, damaged cells, or irritants (Das et al., 2014). Overproduction of reactive oxygen species (ROS) during inflammatory process induces cytokines (TNFα, IL1β, IL6) release and pro-inflammatory enzymes activation (Phospholipase, cyclooxygenases, lipoxygenase) (Manouze et al., 2017). Most treatments for inflammatory diseases use non-steroidal anti-inflammatory drugs (NSAIDs).

Table 5. Mortality rate in acute oral toxicity of *C. nucifera* and *C. papaya* extracts.

Extracts	1st Test		2nd Test	
	Mortality	Mortality rate (%)	Mortality	Mortality rate (%)
Control	0/3	0	0/3	0
CN M	0/3	0	0/3	0
CN A	0/3	0	0/3	0
CP M	0/3	0	0/3	0
CP A	0/3	0	0/3	0

CN M: *C. nucifera* methanolic extract; CN A: *C. nucifera* aqueous extract; CP M: *C. papaya* methanolic extract; CP A: *C. papaya* aqueous extract.

The extracts have more affinity to inhibit the activity of cyclooxygenase 2 than that of COX-1. According to Bacchi et al. (2012), the extracts can be classified in the 2nd group of NSAIDs (capacity to inhibit COX-1 and COX-2 with a preferential selectivity toward COX-2). It is generally thought that their principal mechanism of action is the inhibition of cyclooxygenase (COX-2), the enzyme responsible for biosynthesing the prostaglandins and thromboxane (Jordan and White, 2001). However, this class of drug contains many side effects (Scheiman, 2016). It seems that simultaneously inhibiting COX and LOX, and therefore decreasing the production of leukotrienes and prostaglandins may offer clinically relevant advantages over COX inhibition (Bacchi et al., 2012). The present study showed that the extracts of *Cocos nucifera* and *Carica papaya* roots inhibited the activity of 15-LOX, $sPLA_2$, COX-1, and COX-2, the key enzymes in the formation of eicosanoids (inflammation mediators) from arachidonic acid. The flavonoids, saponins and tannins might be responsible in part for the observed anti-inflammatory effects (Das et al., 2014; Kamau et al., 2016).

Acute oral toxicity

The results of acute toxicity study concerning mortality rate of extracts are presented in Table 5. Acute oral toxicity evaluation reported that no mortality was observed in mice with single dose of 2000 mg / kg right through the 14 days experiment. None of the extracts produced notable changes in behavior during the time of observation. The same observation was made in both steps of the study. These results suggest that aqueous and methanolic extracts from *C. nucifera* and *C. papaya* were classified in class 5 of toxicity and estimated the median lethal dose LD_{50} at 5000 mg / kg, according to acute toxicity class method of OECD guideline 423 (OECD, 2001). The extracts were classified to belong to substances with a low acute oral toxicity according to the Globally Harmonized System of Classification and Labeling of Chemicals of the United Nations (ONU, 2017).

CONCLUSION

The present study demonstrated that methanolic and aqueous extracts of the roots from *C. nucifera* L. and *C. papaya* L. are non-toxic substances, and possess anti-inflammatory and antioxidant effects. These plants contain flavonoids, saponins and tannins. This study product provides scientific data for traditional use of *C. nucifera* and *C. papaya* to treat inflammatory diseases.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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