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Evaluation of Effect of Dietary Supplementation with *Dacryodes edulis* G. Don Pulp Oil on Serum Lipid Parameters in Wistar Albino Rats

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Abstract: The pulp of *Dacryodes edulis* G. Don which is rich in oils is commonly consumed in Nigeria when in season. The effect of diet supplementation with edulis fruit pulp oil on body lipid parameters was evaluated in male wistar albino rats. *D. edulis* oil was extracted in n-hexane. The test diet was compounded using the oil extract (10%), whereas the control animals were kept on control diet formulated with groundnut oil (10%). After six weeks, the animals were anaesthetized with chloroform and blood samples collected through cardiac puncture for the determination of serum lipid profile. Results revealed that *D. edulis* fruit pulp oil did not cause any significant ($p > 0.05$) alterations in serum total cholesterol, LDL-cholesterol and triacylglycerol. The total amount of lipids present in the serum was increased by 33.3%, whereas the quantity of liver lipids decreased by the same factor (33.3%). Insignificant ($p > 0.05$) increases in the weights ($\text{g } 100 \text{ g}^{-1} \text{ BW}$) of the liver (2.91 ± 0.17 to 3.38 ± 0.25), kidney (0.36 ± 0.06 to 0.40 ± 0.02) and heart (0.32 ± 0.02 to 0.33 ± 0.04) were observed in the test group. No significant change ($p > 0.05$) in the average body weight of the test animals was recorded. HPLC analysis of *D. edulis* oil showed that it contained palmitic acid (48.7%), linoleic acid (28.6%), oleic acid (12.9%), stearic acid (5.0%), lauric acid (2.2%), linolenic acid (1.7%) and myristic acid (0.9%). The peroxide value of the oil was 0.00. Prolonged intake of *D. edulis* fruit pulp oil may induce adverse effects on the body organs, even though the body lipid profile remains unaltered.

Key words: *Dacryodes edulis*, serum lipids, arteriosclerosis, dietary lipids, African pear

INTRODUCTION

The African pear (*Dacryodes edulis* G. Don) fruit is one amongst the many indigenous tropical fruits greatly cherished and appreciated for its pulp. The plant belongs to the Burseraceae family and possesses many medicinal and nutritional properties. In Nigeria, when in season, the fruit pulp which is contained in a pod is traditionally consumed raw or after tenderization in hot water, hot ash or roasted (Isaac and Ekpa, 2009). Sometimes, it may be enjoyed with roasted or boiled corn (Iyawe *et al.*, 2007).

Several studies have focused on the chemical composition of the *D. edulis* fruit pulp. The percentage fatty acid composition of the lipid yield includes palmitic acid (30- 62), linoleic acid (15-24), oleic acid (18-60), stearic acid (1.3-5.5) depending on the geographical location of the tree (Mbofung *et al.*, 2002; Kinkela *et al.*, 2006; Ikhuoria and Maliki, 2007; Ajibesin, 2011). Nutritionally important mineral elements such as phosphorus, sodium, zinc and manganese are also found in Eastern Nigeria varieties (Ajayi and Adesanwo, 2009). The essential oils of the pulp as reported by Jitrovetz *et al.* (2004); included α -pinene, β -pinene and

myrcene. Bioactive compounds such as saponins, tannins, alkaloids and flavonoids have been identified in various parts of the plant (Okwu and Nnamdi, 2008) and these have been suggested to be responsible for the enormous ethno-medical applications of *D. edulis* in the treatment of skin diseases, inflammation, bacterial and fungal infections (Okwu and Nnamdi, 2008; Ajibesin, 2011).

The implication of high total cholesterol, Low-Density-Lipoprotein (LDL)-cholesterol, triacylglycerol and low High-Density Lipoprotein (HDL)-cholesterol in the development of cardiovascular disorders such as hypertension, arteriosclerosis, stroke and heart failure can never be over emphasized (Ghasi *et al.*, 2000). There has been tremendous increase in the use of functional foods and, or nutraceuticals due to their beneficial effects on human health. For instance, cardiovascular activity of *D. edulis* oil in rats was reported by Ajibesin (2011). Oil extracted from prickly pear seeds oil has been found to exhibit hypoglycaemic and hypocholesterolemic effects (Ennouri *et al.*, 2007). Changes in the lipoprotein composition of the plasma or serum could be attributed to the type of fat ingested in the diet. The present study was therefore designed to

investigate the effect of continuous dietary intake of *D. edulis* fruit pulp oil (DFPO) extracted in n-hexane on serum lipid parameters of Wistar albino rats (*Ratus norvegicus*).

MATERIALS AND METHODS

Plant materials: Healthy African pear (*D. edulis*) fruits were collected from Abagana, Anambra State, Nigeira, in January, 2011. They were identified and authenticated of by Prof. R. Okigbo, of the Department of Botany, Nnamdi Azikiwe University, Awka. Voucher specimen (herbarium No. N.A.U.H No 112) was prepared and deposited in the herbarium.

The fruits were washed and the fruit pulps separated from the seeds, air-dried at room temperature, wrapped in polyethylene bags and stored in desiccators until needed. The dried fruit pulp was later pulverized using a grinding machine. Three hundred gram quantity of the sample was transferred into a thimble and oil content extracted for 2 h using normal hexane *in vacuo* with soxhlet apparatus. At the end of the extraction, the extracting solvent (n-hexane) was evaporated using rotary evaporator leaving the concentrated oil sample which was used for the experiments. The percentage oil yield was determined gravimetrically.

Animal materials: Sixteen apparently healthy Wistar albino rats weighing between 100.0 to 120.0 g were purchased from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were kept in cages in the animal house of the Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, for one week to allow for acclimatization.

Chemicals used: Analytical chemicals were used and all were products of British Drug House (BDH), Poole England. Triacylglycerol total cholesterol, LDL-cholesterol and HDL-cholesterol diagnostic Kits were obtained from Randox Laboratories Ltd., Ardmore, U.K.

Experimentation: The animals were divided into two groups of eight rats each in accordance with their weights and housed in separate cages.

The first group was fed with the control diet compounded with 10% groundnut oil, whereas the second group of rats was kept on the test diet compounded with 10% *D. edulis* fruit pulp oil. The chemical compositions of both diets were as illustrated in Table 1. The body weights of animals and feed intake were recorded at two days interval for a period of six weeks.

Table 1: Composition of the control and *Dacryodes edulis* fruit pulp oil diets (g 100 g⁻¹ diet)

Parameters	Control	DFPO
Vitamin mixture ^a	1	1
Salt mixture ^b	4	4
<i>Arachis hypogea</i> (groundnut) oil	10	-
<i>Dacryodes edulis</i> fruit pulp oil (DFPO)	-	10
Casilan ^c	26	26
Coru starch	59	59

Vitamin mixture according to Rao *et al.* (1991), Salt mixture according to Rao *et al.* (1991), This diet (100 g) containing 26 g of casilan provided 18.5% of the protein, DFPO *Dacryodes edulis* fruit pulp oil

The daily feed intake was calculated using the equation:

$$\text{Feed in take (g)} = \left[\frac{\text{Da} - \text{Db}}{8} \right] \frac{b}{2}$$

where, Da is the amount placed in the bin feed and Db is the amount remaining after 2 days. 8 corresponds to the number of animals in each cage.

All the animals were allowed free access to feed and drinkable water *ad libitum*.

At the expiration of six weeks period of feeding, the animals were anaesthetized with chloroform and blood samples collected through cardiac puncture for the determination of serum lipid profile. The sera samples were obtained as the supernatant after centrifuging the coagulated blood samples at 3,000 r.p.m for 15 min. Serum total cholesterol, LDL-cholesterol, HDL-cholesterol and triacylglycerol levels were determined using diagnostic kits from Randox Laboratories, U.K.

Lipid extraction from serum and liver: The total lipids of sera samples (500 µL) and liver (2 g) were extracted with chloroform: methanol (2:1, v/v) according to the method of Folch *et al.* (1957). The quantities of total lipids in the sera and liver samples were measured gravimetrically after evaporating the extracting solvent in the sera and liver extracts.

Physicochemical studies: The physicochemical properties of the DFPO were determined immediately by the AOAC (1975).

Fatty acid composition of the oil: Fatty acid profile of the oil was obtained using a high-performance-liquid chromatographic technique (HPLC).

Proximate composition of the diets: The proximate composition of both control and test diets were determined using the method of AOAC (1984).

Statistical analysis: Arithmetic mean and standard error of mean were calculated and all the data obtained were analyzed statistically using Analysis of Variance (ANOVA). Statistical analyses were made by a SPSS for Windows version 13.0 packaged statistics program. All results represented were Mean±Standard Error of mean (SEM) of six determinations.

RESULTS AND DISCUSSION

Findings from our study showed that *Dacryodes edulis* (G.Don) pulp yielded a pale yellow oil (26.4±0.81% yield) with an agreeable odour. The oil existed in the semisolid state at laboratory temperature of 29°C. The percentage oil yield of 26.4 indicates that the pulp is a rich source of lipids. This is comparable to other reports by Lam *et al.* (1987) and Ajibesin (2011). Supplementation of DFPO (10%) into the diet did not cause any significant difference in body weight gain between the control and the test groups since the p. value (0.11) was greater than 0.05. The average body weight increased from 183.51±2.3g to 190.47±0.6g after six weeks of feeding (Table 2).

The type of lipids ingested in the diet could provoke the deposition of fats in the body tissues and for this reason we also estimated the total quantities of extractable lipids in the serum and liver of the experimental animals. Insignificant (p>0.05) variations in the amount of serum and liver lipids were observed after the six weeks duration of feeding. Also, the relative weights of the vital organs (the liver, pancreas, kidney and heart) were not statistically different irrespective of whether the animals were fed with groundnut oil or DFPO (Table 2). Slight but insignificant (p>0.05) increases in the weights (g 100 g⁻¹ BW) of the liver (2.91±0.17 to 3.38±0.25), kidney (0.36±0.06 to 0.40±0.02) and heart (0.32±0.02 to 0.33±0.04) were observed in the test group. This implies that accumulation of lipids in these organs, particularly the liver which plays a pivotal role in lipid metabolism, as a result of the supplementation of the diet with DFPO was negligible.

Also supporting the above inferences is the fact that supplementation of the diet with DFPO did not produce any remarkable alterations in the serum total-cholesterol, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol and triacylglycerol levels of the animals (Table 3). All the observed values were within the normal ranges for total cholesterol (g L⁻¹) (0.69±0.07), LDL-cholesterol (g L⁻¹) (0.21±0.03), HDL-cholesterol (g L⁻¹) (0.37±0.05) and triacylglycerols (g L⁻¹) (0.56±0.07) in the serum of adult albino rats (Alonso *et al.*, 2001; Gaiva *et al.*, 2003).

Table 2: Body weights, feed intakes and relative weights of some vital organs after six weeks of feeding in control and DFPO groups

Parameters	Control	DFPO
Average initial weight (g)	229.07±1.70	183.51±2.300
Average final weight (g)	261.32±0.90	190.47±0.600
Average weight gain (g rat ⁻¹)	32.25±3.80	6.94±1.500
Average feed intake (g rat day ⁻¹)	24.47±2.30	21.82±0.900
Average relative weights of organs		
Liver (g 100 g ⁻¹ BW)	2.91±0.17	3.38±0.250
Pancreas (g 100 g ⁻¹ BW)	0.37±0.03	0.35±0.060
Kidney (g 100 g ⁻¹ BW)	0.36±0.06	0.40±0.020
Heart (g 100 g ⁻¹ BW)	0.32±0.02	0.33±0.040
Quantitative estimate of serum and liver lipids		
Serum lipid (g)	0.03±0.005	0.05±0.015
Liver lipid (g)	0.03±0.007	0.02±0.004

Each value represents the mean of eight rats±standard error of mean. No significant difference between groups was noted. p>0.05, DFPO *Dacryodes edulis* fruit pulp oil

Table 3: Serum lipid profile of rats fed control and DFPO diets

Parameters	Control	DFPO
Total cholesterol (g L ⁻¹)	0.69±0.07	0.75±0.02
LDL-Cholesterol (Lg L ⁻¹)	0.21±0.03	0.23±0.02
HDL-Cholesterol (g L ⁻¹)	0.37±0.05	0.39±0.04
VLDL-Cholesterol (g L ⁻¹)	0.11±0.01	0.13±0.03
Triacylglycerol (g L ⁻¹)	0.56±0.07	0.64±0.05
*Atherogenic index	0.87±0.12	0.92±0.07

Values are Mean±SEM of eight observations. No significant difference between groups was found at 95% level of significance. p>0.05 for all figures in the same row, *Atherogenic index (A.I) = Total cholesterol-HDL-cholesterol, HDL-cholesterol, Deguchi and Ogata (1991), DFPO *Dacryodes edulis* fruit pulp oil

Table 4: Chemical properties of DFPO extracted with n-hexane

Parameter	Observation
Acid value	18.51±2.38
Iodine value	50.32±0.63
Peroxide value	0.000000
Saponification value	84.15±5.87

Values are Mean±SEM of eight observations, DFPO *Dacryodes edulis* fruit pulp oil

Table 4 shows the chemical properties of the *D. edulis* fruit pulp oil. Acid value is a parameter that is used to indicate the suitability of oil for consumption. The acid value of DFPO was 18.51±2.38. This is similar to that of the conventional palm kernel oil, 14.04±0.022 (Akubugwo and Ugbogu, 2007; Igbum *et al.*, 2012) and *Landolphia owariensis*, 15±0.38. (Pearson, 1976). High iodine value of 50.32±0.63 observed in this study is a clear indication of high level of unsaturation of the fatty acid components of DFPO. High-performance-liquid chromatographic (HPLC) analysis of the oil also revealed the presence of appreciable quantities of oleic acid (12.94%) and linoleic acid (28.57%). Omega-3 fatty acid, linolenic acid (1.65%) was also detected (Table 5). The health benefits associated with the consumption of oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3) have been well documented (Ennouri *et al.*, 2007, 2005; Chattopadhyay and Bandyopadhyay, 2005; Ezekwesili *et al.*, 2010). particularly in alleviating cardiovascular, inflammatory and autoimmune disorders.

Table 5: Fatty acid profile of DFPO extracted in n-hexane as given by HPLC

Carbon skeleton	Name	Percentage composition
C12:0	Lauric acid	2.20
C14:0	Myristic acid	0.94
C16:0	Palmitic acid	48.74
C18:0	Stearic acid	4.96
C18:1	Oleic acid	12.94
C18:2	Linoleic acid	28.57
C18:3	Linolenic acid	1.65

DFPO *Dacryodes edulis* fruit pulp oil

However, since the iodine value is also a parameter that indicates the capacity of the oil to go rancid (Amoo *et al.*, 2004), adequate protection should be ensured during storage of DFPO in order to prevent lipid peroxidation.

The peroxide value serves as an index to assess the deterioration of oils. Freshly prepared oils usually have values that are equal to or less than 10, whereas higher ranges (20-40) indicate rancidity of the oils (Akubugwo and Ugbogu, 2007). The peroxide value of DFPO was 0.00. This may suggest that DFPO have antioxidants that resist rancidity by preventing lipid peroxidation.

The iodine value and fatty acid profile of DFPO are very similar to those found in the conventional oil palm oil (from *Elais guineensis* variety). The oil contains approximately equal amounts of saturated (50.85%) and unsaturated (49.14%) fatty acids Lam *et al.* (1987).

CONCLUSION

Data obtained from this study demonstrated that *Dacryodes edulis* (G. Don) fruit pulp is a high oil-yield fruit producing nutritionally, biochemically and medicinally important oil. Supplementation of animal diet with this oil did not cause any significant alteration in the serum lipid profile of the experimental animals.

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