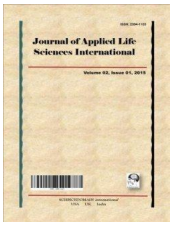

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The Effect of *Dacroydes edulis* (African Pear) Pulp Oil Extract on Serum Lipid Parameters in Male Albino Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author COJO designed and supervised the work. Author CCD wrote and edited the manuscript. Author HCCM edited the manuscript. Authors ICM and VNO managed the literature searches, carried out the laboratory analysis and did the statistical analysis. Authors SOM and VNO managed the literature search. All authors approved the final manuscript.

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ABSTRACT

Aims: This work aimed at evaluating the effects of Dacroydes edulis pulp oil extract on serum lipid profile of male-albino Wistar rats at various concentrations. This was done by assessing serum total cholesterol (TCHOL), high density lipoprotein (HDL) and low density lipoprotein (LDL) levels. This is in order to widen its utilization for the treatment and control of cardiovascular diseases.
Study Design: The research was carried out by grouping the rats into four groups of five rats each (groups 1-4). The groupings were according to the doses of the extract given.
Methods: The oil extract was done using n-lexane in soxhlet apparatus at room temperature and

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the solvent evaporated using rotary evaporation (model TT22, USA) at 50°C. Twenty male albino Wistar rats of 180-200 g were grouped into 4. Groups 1 were the control while groups 2,3 and 4 were given, 5 mg/kg/d, 10 mg/kg/d and 20 mg/kg/d of the pulp oil extract for 21 days. Blood samples were collected for serum lipid profile via ocular puncture using heparinized capillary tubes. Serum lipid profile Levels were analyzed using VIS-UV spectrophotometer (model 752, China). Results were analyzed using SPSS version 21 statistical software using ANOVA analysis.

Place and Duration of Study: This study was conducted at the Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University-Awka, Nigeria. The study lasted for forty days.

The Results: The results analyzed so far showed that among all the parameters tested for (total cholesterol, high density lipoprotein and low density lipoprotein, only serum high density lipoprotein showed a significant increase ($P<0.05$) in levels in the test groups (groups 2-4) when compared to the control group (40.31 $\pm$ 15.4 mg/kg/g). Those given 20 mg/kg/d of the pulp oil extract (group 4) had the highest level of serum high density lipoprotein (212.12 mg/kg/d) among all the treatment groups. ($P< 0.05$). The animals also recorded increased in weight at 21 days of treatment.

Conclusion: The results from this study suggest that the pulp oil extract of *Dacryodes edulis* might have increased high density lipoprotein in a dose dependent manner. The pulp oil extract may, therefore, be useful in the treatment and management of some cardiovascular diseases.

Keywords: Dacroydes edulis; lipid profile; pulp extracts; African pear and n-hexane.

1. INTRODUCTION

1.1 Background of the study

According to [1,2,3], the use of plants by man as a treatment for diseases has been going before civilization era. Local people in villages believe so much in the efficacy of herbal drugs [4,5]. There are many trees and shrubs in Nigeria with high medicinal and nutritional values. There is now increase in the utilization of traditional medicine due to its low cost and availability. The natural product solution may be from plant species, essential oils and fruits.

Fig. 1. A photograph of the *Dacroydes edulis* plant obtained from Nnewi, Anambra State, Nigeria

Many species of dacryodes are underutilized and have been used in folk medicine to treat diseases such as anaemia, malaria, headache, fever and skin diseases [6,7]. Dacryodes have a long history of medicinal use in Africa. Many traditional medicine practitioners use these species to treat diseases [8,9]. According to [10], the bark, leaves, resin and edible fruits of *Dacryodes edulis* are used to treat headache, fever and malaria. *Dacryodes edulis* have been reported to contain high amount of lipids and proteins [11]. This study, therefore, aimed at evaluating the effects *Dacryodes edulis* pulp-oil extracts on the serum lipid parameters which include total cholesterol, high density lipoprotein and low density lipoprotein-cholesterol in normal albino Wistar rats.

There has been a global increase in cardiovascular diseases at an alarming rate. Cardiovascular diseases are the world's largest killer, claiming seventeen million lives a year [12]. Some of these cardiovascular diseases can be attributed to the type of food and drug taken. Some of the patients who died of cardiovascular diseases may be that they were not given adequate medical care or that they did not have access to good drugs as a result of cost and availability of the drugs. Hence there is the need to find out an alternative treatment which should be cheaper and easily accessible. So many research works have been done on the various chemical and phytochemical activities of Dacryodes edulis. No research work has been done on its effects on the serum lipid profile of

male albino Wistar rats. This work, therefore, seeks to close this gap. This study aimed to evaluate the effects of Dacryodes edulis pulp oil extract on the serum lipid profile of normal albino Wistar rats. These include total cholesterol (Total). Low density, lipoprotein Cholesterol (LDL-TCHOL) and high density lipoprotein cholesterol (HDL-CHOL).

2. MATERIALS AND METHODS

2.1 Materials

All reagents, kits used were obtained from Randox laboratories limited, the United Kingdom through their sales representative at Ontisha, Anambra State, Nigeria. The materials used include total cholesterol, reagent kits, high density lipoprotein reagent kit, low density lipoprotein reagent kit, standard plastic animal cages, centrifuge machine (model 800 D, China) and weighing balance (AHAUS, China). Others are refrigerator (Haier Thermocool, HTF-319H, China), n-hexane plain specimen bottles, sterile syringes and capillary tubes.

2.2 Methods

2.2.1 Collection of plant samples

Samples of *Dacryodes edulis* (African Pear) were purchased from Ekeamobi Market, Nnewi, Anambra State, Nigeria and transported to the Human Physiology Laboratory, College of Health Sciences, Nnamdi Azikiwe University, for onward analysis. Authentication/identification was done at herbarium unit, Department of Botany, Nnamdi Azikiwe University Awka. The specimen with identification number NAU/H/446 was deposited there for future reference.

2.2.2 Extraction of pulp oil from *Dacryodes edulis*

This was done according to the method described by [13] fruit samples of the plant were washed with distilled water after which the fruits were kept for about 2 h for the water to dry off. A sharp knife was used to cut open the fruit in order to remove the seeds. This was followed by chopping the soft part of the fruit with smaller pieces using a sharp knife. After this, the samples were pulverized, using a homogenizing machine (model HR2011, China). The powdered sample (200 g) was soaked into 1.75 l of n-hexane, poured into a glass container and sealed. The pulp oil was then extracted from the n-hexane in soxhlet apparatus (model R123, England). The extract was concentrated by evaporating the solvent using rotary evaporator (model T22, USA) at the Human Biochemistry Laboratory, Faculty of Basic Medical Science, College of Health Sciences, Nnamdi Azikiwe University-Awka, Nnewi Campus.

2.3 Animal Handling

Twenty male albino Wistar rats about three months old between 110-150 g were obtained from the Animal Facility Unit of College of Health Sciences, Nnamdi Azikiwe University, Nnewi campus. The rats were kept in clean-dry metal cages with 12 h light-dark cycle. The animals were fed on a pellet diet (vital groups) obtained from Giland Cereals Ltd., a subsidiary of UAC Nigeria PLC, Zawana, Jos, Plateau State, Nigeria, through her sales representative at Ekeamobi Market, Nnewi, Anambra State. They were given water and feed ad libitum. Animal care and handling were done according to guideline given by WHO,[14].

2.4 Experimental Design

The research was carried out by grouping the rats into 4 groups of 5 rats each (groups 1-4). The animals were acclimatized for fourteen days. They were grouped as follows:

Group 1: Control

Those that received distilled water with normal rats feed.

Group 2: Those that received 5 mg/kg/bw of *Dacryodes edulis* pulp oil extract orally for 21d.

Group 3: These that received 10 mg/kg/bw of *Dacryodes edulis* pulp oil extract orally for 21d.

Group 4: These that received 20 mg/kg/bw of *Dacryodes edulis* pulp oil extract orally for 21d.

Administration of the extract was done via oral route using oral canular.

2.5 Collection of Blood Samples

This was done via ocular puncture using heperinized capillary tubes and transferred into plain specimen bottles labeled accordingly for the groups. The sample serum was separated from the samples by subjecting them to centrifugation using ultra modern centrifuge (model 90 l,

Alphine Medical, England) at 3000 rpm for 20 min at room temperature.

2.6 Estimation of Serum Lipid Profile

This was done using the method recommended by WHO [14]. The lipid parameters that were estimated includes total cholesterol, high density lipoprotein-cholesterol and low density lipoprotein-cholesterol.

1. Estimation of Serum Total Cholesterol (TCHOL)

Principle:

Total cholesterol was estimated based on the principles that cholesterol undergoes enzymatic hydrolysis and oxidation to form quinonamine from hydrogen peroxide and 4-amino antipyrine in the presence of phenol and peroxidase, thereby developing a colour whose absorbance is measured at 500-546 nm.

Procedure:

Fifteen mills of distilled water were added to cholesterol reagent 1. Ten microliltre (10 µl) each of distilled water, standard and serum were added to test tubes marked blank, standard and test respectively. This was followed by the addition of 1ml of cholesterol reagent 1 to all the tubes. These were mixed thoroughl and incubated for 10 min at room temperature using laboratory incubator (model DNP 9052 A, China). The absorbance of the standard and samples were measured against the reagent blank spectrophotometrically at 500 nm using UV-VIS spectrophotometer (Model 725G China).

$$\text{Conc of Total Cholesterol} = \frac{\text{Abs Test} - \text{Abs Blank}}{\text{Abs STD} - \text{Abs Blank}} \times \text{Conc of STD(mg/dl)}$$

STD = standard

Abs= absorbance

2. Estimation of Serum High Density Lipoprotein

Principle:

This was based on the principle that very low density lipoprotein and low density lipoprotein are precipitated in serum with phosphotungsitic acid (precipitating agent). The high density lipoprotein cholesterol remaning in solution is then measured spectrophotometrically by means of coupled reactions with 4-aminoantipyrine in the presence of cholesterol esterase oxidase and peroxidase to form quinonamine.

Procedure:

One mill of precipitating reagent (B) was diluted with distilled water .Two hundred microlitre (200 µl) each of standard and test were added to test tubes marked standard and tests. Five hundred microlitre (µl) of the diluted precipitants was added to all the tubes and mixed thoroughly. These were incubated for 10 min at room temperature. They were centrifuged for 10 min at 4,000.rpm at room temperature using Ultra Modern Centrifuge machine(model 90 l, Alphine Medical, England). Supernatant was collected. Fresh tubes were labeled blank, standard and test respectively. Hundred microlitre (100 µl) of distilled water was added to the test tube marked blank while 100 µl of supernatant for test was added to test tube marked test. One mill of cholesterol reagent was added to all the tubes and mixed thoroughly. These were incubated for 10 min at room temperature. The absorbance of sample and standard were measured against the reagent blank. spectrophotometrically using UV-VIS Spectrophotometer (model 725G, China). The concentration of high density lipoprotein cholesterol was calculated using:

$$\text{Conc. Of high density lipoprotein cholesterol} = \frac{\text{Abs Test- Abs Blank}}{\text{Abs STD- Abs Blank}} \times \text{Conc of STD(mg/dl)}$$

3. Low density lipoprotein (LDL)

Principle:

Low density lipoprotein undergoes coupled reactions whose colour intensity was measured spectrophotometrically at 500-546 nm.

Procedure:

Two hundred microlitre of each sample was pipetted in a test tube. This was followed by the addition of two hundred microlitres of LDL-Cholesterol reagent (reagent A) to each test tube. These were mixed and allowed to stand for 15 min at room temperature. The tubes were centrifuged for 15 min at 4000 rpm using Ultra Modern Centrifuge machine (model 90l, Alphine Medical England). Fresh tubes were labeled blank, standard and samples. Twenty microlitre

of distilled water was added to the test tube for blank. Twenty mills of each sample supernatant were pipette into the test tube for sample and 20 ml of cholesterol standard was pipette into the test tube for standard. One mill of cholesterol reagent was added to all the test tube, mixed thoroughly and incubated for 30 min at room temperature. The absorbance of the standard and sample was measured at 500 nm against the reagent blank using UV-VIS Spectrophotometer (spectrum 725G, China). The concentration of LDL-Cholesterol was calculated using:

$$\text{Conc. LDL} = \frac{\text{Abs Test}}{\text{Abs STD}} \times \text{Conc of STD} \times \text{dilution factor (mg/dl)}$$

2.7 Statistical Analysis

The data obtained from the lipid profile parameters tested above were statistically analyzed using the one way ANOVA technique with SPSS Version 2016 software at 0.05 level of significance. They were represented as mean ± SEM.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 The effect of pulp oil extract of *Dacryodes edulis* on serum lipid profile of male albino Wistar rats

The results of serum lipid profiles of albino Wistar given pulp oil extract of *Dacryodes edulis* were presented in table 1. Results from table 1 show that there was significant increase only in the HDL level ($P<0.05$) for all the test groups (groups 2 to 4) when compared with the control group (40.31±15.4 mg/dl). There was no significant increase in the levels of serum total cholesterol and LDL when compared with the control group(113.47±9.65 and 84.62±22.79 mg/dl)

3.1.2 The effect of pulp oil extract of D*acryodes edulis* on body weights of male albino Wistar ratss

The final body weights of all the rats' groups were significantly higher($P<0.05$) than the initial body weight as can be seen in Table 2.

3.2 Discussion

The results of this study showed that the serum total cholesterol and low density lipoprotein cholesterol (LDL- Cholesterol) levels of the all the groups of the rats given *Dacroydes edulis* pulp oil extract were not significantly higher or lower when compared with the control group (group 1) and within the test groups ($P<0.05$). This may be suggesting that the pulp oil extract of this plant (*Dacryodes edulis*) may not have affected the serum total cholesterol and low density lipoprotein cholesterol levels of those rats given the extract. This agrees with the similar reports of [15,16,17,18] on the effects of *Dacroydes edulis* pulp oil extract on serum lipid profile levels of albino rats. According to [16], high levels of total cholesterol, low density lipoprotein are risk factors for atherosclerosis. However the results also showed significant increase in the level of serum high density lipoprotein of all the groups of rats given the extract when compared with the control with the group given the highest dose (20 mg) having the highest increase while the group given the lowest dose (5 mg) having the least increase (Table 1). This is a pointer that the extract may be a source of good cholesterol in the body and may be good for the heart since it increased the good cholesterol (HDL-Cholesterol). This is supported by the findings of [18,19,20,21]. High density lipoproteins oppose atherosclerosis directly by removing cholesterol from foam cells by inhibiting the oxidation of low density lipoproteins and by limiting the inflammatory processes that underlie atherosclerosis [22]. High density lipoproteins also have antithrombotic properties. Thus, HDL-cholesterol interrupts the process of atherogenesis at several key stages. Several medicinal plant species have been reported to have shown a dose dependent effect on the treatment of abnormal serum lipid profile [23,24]. The highest increase in serum HDL cholesterol witnessed at the highest dose (20 mg of extract) which was followed by the middle dose (10 mg of extract, Table 1) is suggesting that the effects of this extract on the serum HDL-cholesterol levels of these rats are dose-dependent. This agrees with the reports of [15,16].

According to [16,25,26], prevention of atherosclerosis is aimed at reducing total cholesterol, low density lipoprotein cholesterol and increasing high density lipoprotein cholesterol. According to studies done by [27] on the phytochemical screening of the extract of the plant, the pulp oil extract of the plant contained flavonoids and tannins, which have been reported by [28] to be responsible for lowering serum total cholesterol, triglycerides, low density lipoprotein cholesterol and increasing serum level of high density lipoprotein cholesterol in

Table 1. The effect of pulp oil extract of *Dacroydes edulis* on serum lipid profiles

Groups	TCHOL (Mg/dl)	HDL (Mg/dl)	LDL (Mg/dl)
Group 1 (Control)	113.47±9.65	40.31 ±15.47a	84.62±22.79
Group 2 (3 Mg ext)	137.52±20.65	136.77±6.24b	127.39± 26.05
Group 3 (10 Mg/ext)	171.19±36.18	169.69±11.33c	86.11±5.60
Group4 (20 Mg/Ext.)	158.27±26.98	212.12±2.14d	127.68±25.43

All results were analyzed using one way ANOVA at P<0.05 level of significance. The results were represented as mean ± SEM of triplicate determination. Values in the same column bearing a different letter are significant. Results were analyzed using ANOVA analysis

rats. Generally, plants are reported to exhibit lipid lowering activity are rich in flavonoids and tannins, which play a significant role in the mobilization and metabolism .

The results of the body weights analyzed showed that there was a significant increase in the body weight of the rats in all the groups (groups 2 to 4) before and after administration of the extracts (table 2, P<0.05). This agrees with the findings of [15] who found out that there was an increase in the average weights of rats after administration when compared with the initial weights of the rats given the same pulp-oil extract (*Dacroydes edulis*).

Table 2. The effect of pulp oil extract of *Dacroydes edulis* on the body weights of male albino Wistar rats

Groups	Body weights
Group1	
Initial	125.00 +6.45a
Final	155.00+6.45b
Group2	
Initial	127.50 +4.78c
Final	157.50+2.50d
Group3	
Initial	132.50+8.53e
Final	170.00+4.08f
Group4	
Initial	125.00+2.88g
Final	160.00+4.08h

Data was analyzed using student dependent t-test at P<0.05 level of significant. The results were represented mean ± SEM of triplicate determination. Values in the same column bearing different letters are significant.

4. CONCLUSION

There were no significant changes so far on the level of serum total cholesterol and low density lipoprotein cholesterol of the rats given the extract. Based on this, therefore, the extract has no effects on the above mentioned cholesterols. The extract increased serum level of high density lipoprotein with those given highest dose (20 mg/kgbw) showing the highest increase followed by the middle dose (10 mg/kgbw). The treatment is, therefore, dose dependent and the plant's extract may be sources of good cholesterol.

ETHICAL APPROVAL

This work was approved by the ethical committee of College Health Sciences, Nnamdi Azikiwe University-Awka, Nnewi Campus, Anambra State, Nigeria.

COMPETING INTERESTS

The authors declare that no competing interests exist.

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