

Effect of Calamari Oil on Lipid Profile of Streptozotocin Induced Diabetic Rats

U. J. O. Orji^{1*}, H. Brown¹, E. O. Nwachuku¹ and N. Boisa²

¹Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.

²Department of Chemistry, Rivers State University, Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author NB designed the study. Author HB performed the statistical analysis. Author EON managed the analyses of the study. Author UJOO managed the literature searches and wrote the protocol and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CA/2021/v10i330171

Editor(s):

(1) Dr. Jagadeesh Kalavakunta, Michigan State University and Western Michigan University, USA.

Reviewers:

(1) Augustine Ikhueoya Airaodion, Federal University of Technology Owerri, Nigeria.

(2) Ahmet Gökhan Akkan, Bezmialem Vakif University, Turkey.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/69795>

Original Research Article

Received 05 April 2021

Accepted 10 June 2021

Published 22 June 2021

ABSTRACT

Aim: The aim of this study was to assess the effect of Calamari Oil on lipid profile levels in diabetes streptozotocin induced diabetic Rats.

Study design: An experimental study.

Place and duration of study: Animal House, Department of Applied and Environmental Biology, Rivers State University, Port Harcourt and University of Port Harcourt Rivers State, Nigeria, between February 2020 and August 2020.

Methodology: Thirty Six (36) albino rats were purchased and allowed to acclimatize for two (2) weeks in the laboratory at the animal farm house of the Department of Animal and Environmental Biology, Rivers State University. They were fed the normal rat feed (Chow feed) and water was allowed *ad libitum*. The rats were weighed and randomly grouped into six (6) groups with six rats in each group. Group 1 (Negative control) was placed on normal diet while groups 2 to 6 were placed on a high fat diet (HFD) prior to the induction with Streptozotocin to achieve diabetes and the animals were treated according to their groupings for four weeks by means of oral gavage. The dose of Calamari Oil administered to the rats was extrapolated from human doses. The high fat diet was prepared by mixing the animal feed (Chow diet) with margarine in a ratio of 3:1. After each

*Corresponding author: E-mail: uzoamakairoemeh@yahoo.com;

period of treatments, blood samples were collected from the rats at the end of the treatments via cardiac puncture by anaesthetizing the rats with chloroform after a six (6) hour fast. Fasting blood glucose was determined using the Glucose Oxidase method, lipid profile was analysed spectrophotometrically and Atherogenic coefficient (AC) and Castelli ratio index-1 level (CRI-1) were calculated. The GC–MS analysis of bioactive compounds from Calamari Oil was done using Agilent Technologies GC systems with GC-7890A/MS-5975C model. Data generated were analysed using SPSS version 22.0 of windows statistical package. Results were considered statistically significant at 95% confidence interval ($p < 0.05$).

Results: The results showed that after week 1 - 4 of exposure, the mean TG (Triglyceride) value of the Negative control group (NC), Positive control (PC) group, diabetic groups exposed for weeks 1, 2, 3 and 4 expressed in mg/dl were 130.89 ± 2.52 , 174.94 ± 3.11 , 166.64 ± 1.95 , 160.61 ± 0.60 , 153.37 ± 2.24 and 141.62 ± 0.99 respectively. Mean TC (Total cholesterol) value of the NC, PC group, diabetic groups exposed for weeks 1, 2, 3 and 4 expressed in mg/dl were 160.93 ± 2.99 , 194.96 ± 2.09 , 188.18 ± 1.41 , 180.63 ± 0.59 , 169.96 ± 1.47 and 159.71 ± 1.43 respectively. HDL (High density lipoprotein) value of the NC, PC group, diabetic groups exposed for weeks 1, 2, 3 and 4 expressed in mg/dl were 83.05 ± 2.96 , 65.68 ± 1.78 , 66.97 ± 1.28 , 72.75 ± 0.52 , 76.35 ± 0.77 and 78.94 ± 0.86 . Mean LDL (Low density lipoprotein) value of the NC, PC group, diabetic groups exposed for weeks 1, 2, 3 and 4 expressed in mg/dl were 51.7 ± 3.21 , 94.3 ± 0.48 , 88.15 ± 1.08 , 75.76 ± 0.62 , 62.95 ± 0.94 and 52.46 ± 0.59 respectively. The mean non- HDL cholesterol value of the NC, PC group, diabetic groups exposed for weeks 1, 2, 3 and 4 expressed in mg/dl were 159.93 ± 2.99 , 193.96 ± 2.09 , 187.18 ± 1.41 , 179.63 ± 0.59 , 168.96 ± 1.47 and 158.71 ± 1.42 respectively. Also, mean cholesterol ratio value of the NC, PC groups, diabetic groups exposed for weeks 1, 2, 3 and 4 expressed in mg/dl were 1.94 ± 0.07 , 2.97 ± 0.06 , 2.81 ± 0.04 , 2.48 ± 0.02 , 2.22 ± 0.01 and 2.02 ± 0.01 respectively.

Conclusion: Type 2 diabetes is associated with dyslipidemia and as such the treatment with the Calamari Oil in the treated groups had positive effect on lipid profile markers with TG, TC, LDL, AC, CRI-1 decreasing and HDL increasing with increase in duration of time.

Keywords: Calamari oil; lipid profile; diabetes induced rats.

1. INTRODUCTION

Diabetes is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycaemia is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [1]. Diabetes mellitus is a major source of mortality and morbidity along with being an economic menace all over the world. About 422 million people worldwide have diabetes, particularly in low and middle-income countries, and 1.6 million deaths are directly attributed to diabetes each year. With an 8.5% global prevalence of diabetes in 2014, various estimates suggest that the number of affected people will rise from 422 million to 642 million in the world by 2040 [2]. Diabetes is an important public health problem, one of the priority non-communicable diseases (NCDs). Over the past few decades, there has been a steadily increasing number of cases and prevalence of diabetes. Evidence suggests that non-communicable diseases of which diabetes is one of, currently contributes substantially to the

burden of mortality and morbidity in adults. Age-specific levels of diabetes and hypertension in many urban areas of Sub-Saharan Africa are as high as, or higher than those in most Western European countries. In most developed communities the peak of occurrence falls in the age group of 65 years or older, whereas in developing countries it is in the age group 45 to 64, and in Sub-Saharan Africa it is in the age groups 20 to 44 and 45 to 64 years. Data from 12 other studies from Sub-Saharan Africa indicate two peak age ranges of 45 to 64 and older than 65 years [3].

Calamari Oil is sourced from squid and contains the highest source of DHA. Compared to any other omega 3 oil, squid oil consists of high levels of Vitamin A and E, which are essential for growth, vitality and the overall development of human beings. Calamari Oil is also known as Calamarine and the DHA present in it is 85% richer than in fish or other marine extracts. Calamari Oil is specifically rich in DHA which is good for the total health, brain, skin, heart, eyes and nervous system [4]. It is the most environmentally friendly and highest source of

Omega-3 fatty acid and has the highest substance/content of DHA at 470mg/g, followed by algal with 350mg/g and then fish with 120mg/g. Diabetes mellitus as a major risk factor for coronary heart disease, blindness, stroke, kidney failure and peripheral arterial disease poses tremendous public health burdens and it has been demonstrated that lifestyle including diet plays a major role in its development, [5]. It is then important to understand the role of specific food and nutrients in the pathogenesis of Diabetes Mellitus.

Omega-3 polyunsaturated fatty acids (n-3 PUFA) include eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) from seafood, and alpha-linolenic acid (ALA, 18:3n-3) from plant sources. Though the relationship between omega-3 polyunsaturated fatty acids (n-3 PUFA) from seafood (eicosapentaenoic acid, EPA; docosahexaenoic acid, DHA) or plant (alpha-linolenic acid, ALA) sources and risk of type 2 diabetes mellitus (DM) is still unclear, High relative consumption of omega-3 (n-3) polyunsaturated fatty acids is thought to be beneficial for a number of chronic diseases [6]. Therefore, the aim of this study was to assess the effect of Calamari Oil on lipid profile levels in diabetes streptozotocin induced diabetic Rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Thirty Six (36) albino rats were purchased and allowed to acclimatize for two (2) weeks in the laboratory at the animal farm house of the Department of Animal and Environmental Biology, Rivers State University. They were fed the normal rat feed (Chow feed) and water was allowed *ad libitum*. Formal consent was not required for the use of the rats because the Department of Animal and Environmental Biology, Rivers State University animal farm house is a research centre where consent has been given for the use of the laboratory animals for experimental study.

2.2 Treatments

Forever Arctic Deep Sea oil (Calamari Oil) was used in treating the diabetes induced rats.

2.3 Diabetes Mellitus Inducing Agent

Streptozotocin was the inducing agent used. The streptozotocin used was purchased from Carbosynth limited, 8 and 9 Old Station Business

Park, Compton, Berkshire RG20 6NE, United Kingdom.

2.4 Study Design

Thirty six (36) rats were weighed and randomly grouped into six (6) groups with six (6) rats in each group. Group 1 (Negative control) was placed on normal diet while groups 2 to 6 were placed on a high fat diet (HFD) prior to the induction with Streptozotocin to achieve diabetes and the animals were treated according to their groupings for four weeks by means of oral gavage.

Group 1: The animals in this group were used as the negative control, they were not induced with Streptozotocin and were not also fed with High fat diet (HFD). They were only fed with the rat feed and water.

Group 2: The animals in this group were induced with Streptozotocin and became diabetic but were not given any treatment.

Group 3: The animals in this group were induced with Streptozotocin, became diabetic and were treated with Forever Arctic Deep Sea oil (Calamari Oil) and were sacrificed after one week.

Group 4: The animals in this group were induced with Streptozotocin, became diabetic and were treated with Forever Arctic Deep Sea oil (Calamari Oil) and were sacrificed after two weeks.

Group 5: The animals in this group were induced with Streptozotocin, became diabetic and were treated with Forever Arctic Deep Sea oil (Calamari Oil) and were sacrificed after three weeks.

Group 6: The animals in this group were induced with Streptozotocin, became diabetic and were treated with Forever Arctic Deep Sea oil (Calamari Oil) and were sacrificed after four weeks.

2.5 Dose Calculation of Arctic Deep Sea Oil

The dose administered to the rats was extrapolated from human doses.

Human daily dose is one tablet of 435 mg twice daily which is 870 mg daily.

Rat dose (mg/kg) = Human dose x 0.018 x 5 [7]
= 870 X 0.018 X 5
= 78.3 mg/kg. This dose was administered mg per kg body weight to the rats.

2.6 Preparation of High Fat Diet

Top Feeds chow diet manufactured by Eastern Premier Feed Mills Ltd Nigeria was used. The high fat diet was prepared by mixing the animal feed (Chow diet) with margarine in a ratio of 3:1 that is 3 parts of the animal feed to 1 part of the margarine. For every 3 grams of the chow diet feed, 1 gram of margarine was added to obtain the High fat diet (HFD). The HFD was sent to the Department of Food Science Technology, Rivers State University to determine the fat content of the feed since a HFD should have a fat percentage level of within 40 – 60%.

2.7 Sample Collection and Storage

Blood samples were collected from the rats at the end of the treatments via cardiac puncture by anaesthetizing the rats with chloroform after a six (6) hour fast. This is in line with the National Institutes of Health (NIH) and the Animal Models of Diabetic Complications Consortium (AMDCC) protocol on the fasting of laboratory animals. Blood sample for fasting blood glucose was put in a fluoride oxalate bottle and in plain bottles for the analysis of Lipid profile (Total cholesterol, Triglyceride and HDL). Samples for fasting blood glucose were analysed within six (6) hours. The sample containers with anticoagulants were mixed thoroughly. Samples for the other parameters were preserved pending the determination time.

2.8 Biochemical Analyses

2.8.1 Determination of fasting blood glucose

Glucose Oxidase method [8] was used in the quantitative estimation of the fasting blood glucose levels. The glucose oxidase kit used was manufactured by Randox laboratories limited, United Kingdom. Glucose oxidase (GOD) catalyses the oxidation of glucose to give gluconic acid and hydrogen peroxide (H₂O₂). The hydrogen peroxide formed reacts with phenol and 4-aminophenazone under the catalysis of peroxidase (POD) to form a red violet or pink substance whose absorbance is read

spectrophotometrically at 520nm. The absorbance is directly proportional to the concentration of the glucose.

2.8.2 Laboratory investigation of lipid profile parameters

All the biochemical investigations were carried out at University of Port Harcourt Rivers State, Nigeria and reagents used were commercially purchased and the manufacturer's standard operating procedures were strictly adhered to.

2.8.3 Determination of total cholesterol (TC)

TC was measured quantitatively by enzymatic method [9] as described by Liquichek, Agappe diagnostics limited Switzerland. The principle is based on the determination of Total cholesterol after enzymatic hydrolysis and oxidation. The indicator quinoeimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. The intensity of colour formed is proportional to the concentration of total cholesterol.

2.8.4 Determination of triglyceride (TG)

TG was measured quantitatively by enzymatic method [10] as described by Liquichek, Agappe diagnostics limited Switzerland. Triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

2.8.5 Determination of high density lipoprotein (HDL) cholesterol

HDLc was measured quantitatively by enzymatic method [11] as described by Liquichek, Agappe diagnostics limited Switzerland. The chylomicrons, Very low density lipoproteins (VLDL) and Low density lipoproteins (LDL) of serum are precipitated by phosphotungstic acid and magnesium ion. After centrifugation, High density lipoprotein (HDL) are in the supernatant. HDL content of supernatant is measured by an enzymatic method.

2.8.6 Determination of low density lipoprotein (LDL) cholesterol and calculation of atherogenic indices

Low density lipoprotein was calculated using Friedewald's formula

$LDL-c = TC - HDL - TG/5$ and the **TC-HDL/HDL** (The mean non- HDL cholesterol value) and **TC/HDL** (mean cholesterol ratio value) were calculated.

2.8.7 Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS analysis of bioactive compounds from Calamari Oil was done using Agilent Technologies GC systems with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length \times 250 μ m in diameter \times 0.25 μ m in thickness of film). Spectroscopic detection by GC-MS involved an electron ionization system which utilized high energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1 mL/min. The initial temperature was set at 50–150 °C with increasing rate of 3°C/min and holding time of about 10 min. Finally, the temperature was increased to 300°C at 10°C/min. One microliter of the prepared 1% of the extracts diluted with respective solvents was injected in a splitless mode. Relative quantity of the chemical compounds present in each of the extracts of was expressed as percentage based on peak area produced in the chromatogram.

2.8.7.1 Identification of chemical constituents

Bioactive compounds extracted from CO were identified based on GC retention time on HP-5MS column and matching of the spectra with computer software data of standards (Replib and Mainlab data of GC-MS systems).

2.9 Statistical Analysis

Data generated were analysed using SPSS version 22.0 of windows statistical package. Results were considered statistically significant at 95% confidence interval ($p < .05$).

3. RESULTS AND DISCUSSION

Bioactive compounds (Zoochemical analysis) of the Calamari Oil used in this study indicate the presence of Phenolic acids, alkaloids, flavonoids, saponins, Tannins in them (Table 1 and Fig. 1).

In the treatment of Type 2 diabetes, heart disease and neurological disorders, bioactive compounds (zoochemicals) have been used and are more effective in the treatment of these diseases and have less side effects than the modern-days medications.

Fasting blood glucose results from this study showed significant difference ($P < .05$) in Fasting blood sugar (FBS) for rats supplemented with Calamari Oil for week one to four (Table 2). The FBS activity when compared with the Negative and positive controls, had a significant variation. In comparison with the positive control, there was a variation in FBS in a decreasing order with an increase in duration at $P < .05$ with the rats supplemented with Calamari Oil studied.

There is porosity of literature on the over the counter Calamari Oil (CO) used in this study. For the over the counter Calamari Oil (CO) group, the results from this study showed significant difference ($P < .05$) in Triglyceride (TG) level for rats supplemented with over the counter Calamari Oil (CO). The TG activity when compared with the Negative and positive controls, had a significant variation. In comparison with the positive control, there was a variation in TG in a decreasing order with an increase in duration at $P < .05$ (Table 2). This result can be due to the presence of proanthocyanin and Naringenin which are bioactive compounds that aids in the decrease of lipid peroxidation markers, inhibiting lipid peroxidation and as such protecting the cardiovascular system and heart [12].

The results from this study showed significant difference ($P < .05$) in Total cholesterol level (TC) for rats supplemented with over the counter Calamari Oil (CO). The TC activity when compared with the Negative and positive controls, had a significant variation. In comparison with the positive control, there was a variation in TC in a decreasing order with increase in duration at $P < .05$. This result can be due to the presence of proanthocyanin and Naringenin which are bioactive compounds that aids in the decrease of lipid peroxidation markers, inhibiting lipid peroxidation and as such protecting the cardiovascular system and heart and also lowers serum cholesterol level [12-13].

The results from this study showed significant difference ($P < .05$) in High density lipoprotein cholesterol (HDLc) for rats supplemented with over the counter Calamari Oil (CO). The HDLc level when compared with the Negative and positive controls, had a significant variation. In comparison with the positive control, there was a variation in HDLc level in an increasing order with an increase in duration at $P < .05$. This can be as result of the presence of some bioactive

compounds such as saponin, tannin and catechin in the oil which lowers levels of HDLs [13].

The results from this study showed significant difference ($P<.05$) in Low density lipoprotein cholesterol level (LDLc) for rats supplemented with over the counter Calamari Oil (CO). The LDLc level when compared with the Negative and positive controls, had a significant variation. In comparison with the positive control, there was a variation in LDLc in a decreasing order with increase in duration at $P<.05$ (Table 3). This can be as result of presence of flavonoids in the oil which prevents LDLs from oxidizing, thereby resulting in the decrease of its concentration [12].

The results from this study showed significant difference ($P<.05$) in Atherogenic coefficient (AC) for rats supplemented with over the counter Calamari Oil (CO). The AC level when compared with the Negative and positive controls, had a significant variation. In comparison with the

positive control, there was a variation in AC in a decreasing order with increase in duration at $P<.05$. This is due to the presence of bioactive compounds such as naringenin, catechin which aids in decreasing lipid peroxidation markers, and proanthocyanidin that protects the heart [12,14].

The results from this study showed significant difference ($P<.05$) in Castelli ratio index-1 level (CRI-1) for rats supplemented with over the counter Calamari Oil (CO). The CRI-1 level when compared with the Negative and positive controls, had a significant variation. In comparison with the positive control, there was a variation in CRI-1 in a decreasing order with increase in duration at $P<.05$. This is due to the presence of bioactive compounds such as naringenin, catechin which aids in decreasing lipid peroxidation markers, and proanthocyanidin that protects the heart [12,14].

Table 1. Qualitative analysis results of the bioactive compounds in CO

Qualitative Analysis	
Component	Result
Proanthocyanin ug/g	+ve
Oxalate ug/g	+ve
Flavan 3 ol ug/g	-ve
Ribalinidine ug/g	+ve
Naringenin ug/g	+ve
Resveratol ug/g	-ve
Phenol ug/g	+ve
Epicatechin ug/g	+ve
Sapogenin ug/g	+ve
Flavonones ug/g	+ve
Phytate ug/g	+ve
Lunamarin ug/g	+ve
Kaempferol ug/g	+ve
Flavone ug/g	+ve
Quinine ug/g	-ve
Anthocyanin ug/g	+ve
Tannins ug/g	+ve
Catechin ug/g	+ve
Naringin ug/g	+ve
Rutin ug/g	+ve
Steroids ug/g	+ve
Isoflavones ug/g	+ve
Spartien ug/g	+ve

Key: +ve = present
-ve = absent

Table 2. Mean glucose and lipid Profile of control groups and group supplemented with Calamari Oil (CO)

Groups/ Parameters	FBS (mmol/l)	TG (mg/dl)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	TC-HDL/HDL	TC/HDL
Negative Ctr	4.36 ± 0.14	130.89±2.52	160.93±2.99	83.05±2.96	51.7±3.21	159.93±2.99	1.94±0.07
DM Not Tr	18.65±0.41	174.94±3.11	194.96±2.09	65.68±1.78	94.3±0.48	193.96±2.09	2.97±0.06
DM + CO1	13.43±0.65	166.64±1.95	188.1±1.41	66.97±1.28	88.15±1.08	187.18±1.41	2.81±0.04
DM + CO2	13.3±0.70	160.61±0.60	180.63±0.59	72.75±0.52	75.76±0.62	179.63±0.59	2.48±0.02
DM + CO3	10.8±0.19	153.37±2.24	169.96±1.47	76.35±0.77	62.95±0.94	168.96±1.47	2.22±0.01
DM + CO4	8.35 ±0.27	141.62±0.99	159.71±1.43	78.94±0.86	52.46±0.59	158.71±1.42	2.02±0.01
P-values	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
F-values	86.16	60.7	63.93	18.31	147.4	63.93	97.21

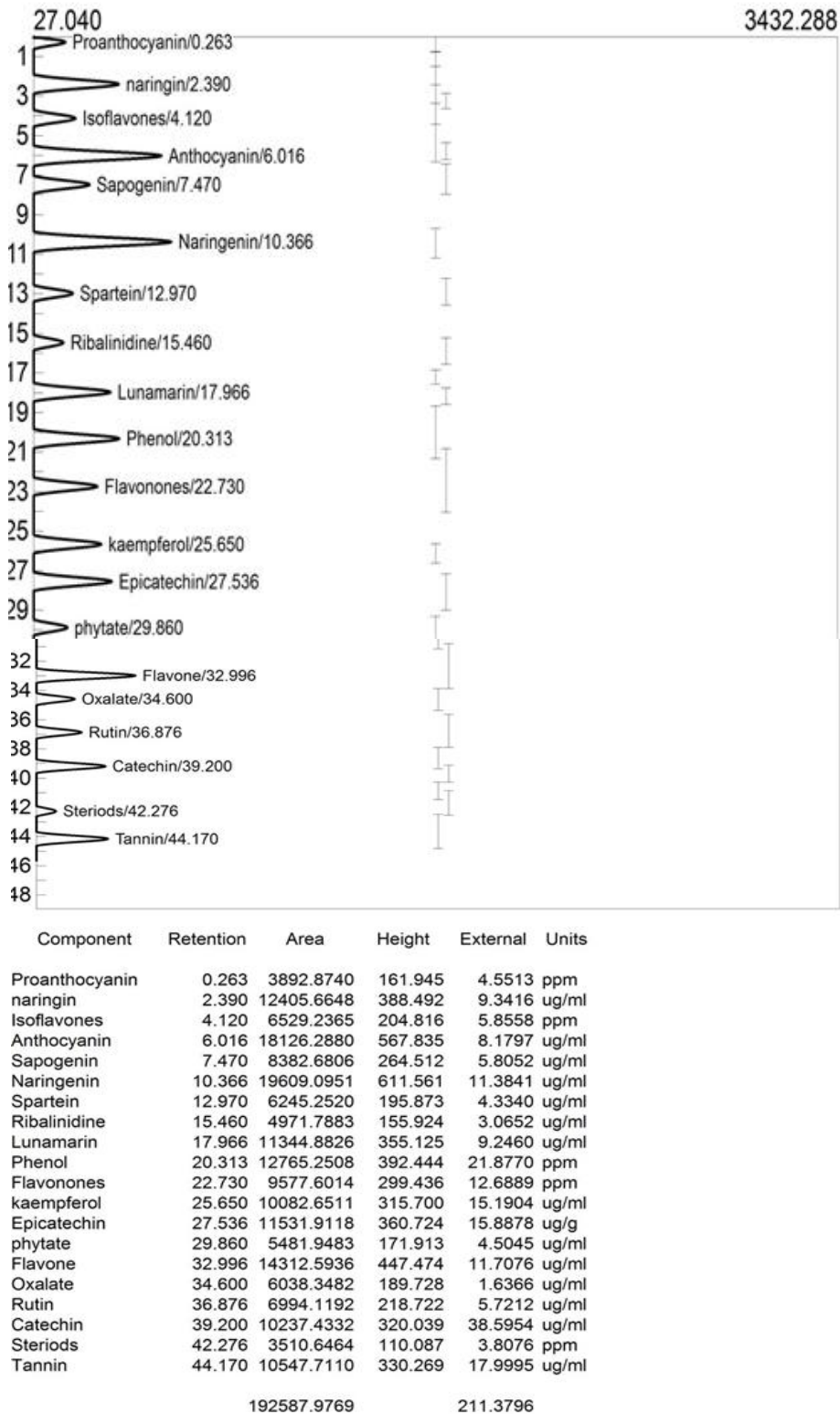


Fig. 1. Quantitative analysis results of the bioactive compounds in CO

Table 3. Post analysis (Post Hoc) of lipid profile of control groups and group supplemented with Calamari Oil (CO) using the Tukey's multiple comparison test

Tukey's Multiple Comparison Test	FBS	TG	TC	HDL	LDL	TC-HDL/HDL	TC/HDL
Negative Control vs DM Group	***	***	***	***	***	***	***
Negative Control vs CO WK1	***	***	***	***	***	***	***
Negative Control vs CO WK2	***	***	***	***	***	***	***
Negative Control vs CO WK3	***	***	*	Ns	***	*	***
Negative Control vs CO WK4	***	*	Ns	Ns	ns	Ns	Ns
DM Group vs CO WK1	***	Ns	Ns	Ns	ns	Ns	Ns
DM Group vs CO WK2	***	***	***	*	***	***	***
DM Group vs CO WK3	***	***	***	***	***	***	***
DM Group vs CO WK4	***	***	***	***	***	***	***
CO WK1 vs CO WK2	NS	Ns	Ns	Ns	***	Ns	***
CO WK1 vs CO WK3	**	**	***	**	***	***	***
CO WK1 vs CO WK4	***	***	***	***	***	***	***
CO WK2 vs CO WK3	**	Ns	**	Ns	***	**	**
CO WK2 vs CO WK4	***	***	***	Ns	***	***	***
CO WK3 vs CO WK4	**	**	**	Ns	***	**	*

Key: Ns – not significant
 *, ** and *** - significant at $p < .05$

4. CONCLUSION

Type 2 diabetes is associated with dyslipidemia and as such the treatment with the Calamari Oil in the treated groups had positive effect on lipid profile markers with TG, TC, LDL, AC, CRI-1 decreasing and HDL increasing with increase in duration of time.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable

ETHICAL APPROVAL

All authors hereby declare that Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

ACKNOWLEDGEMENTS

Authors are grateful to Mr. Barine Toghi Rogers for assisting in taking care of the laboratory animals, Mr. Robinson of University of Port Harcourt for biochemical analysis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. American Diabetes Association (ADA). Diagnosis and classification of diabetes mellitus. *Diab. Care.* 2010;33(1):62-9.
2. World Health Organization. Diabetes Fact Sheet; 2016.
3. Jamison DT, Feachem RG, Makgoba MW, Bos ER, Baingana FK, Hofman KJ and Rogo KO. Disease and mortality in sub-Saharan Africa. 2nd edition. Washington (DC): The International Bank for Reconstruction and Development / The World Bank; 2006.
4. Health calamari oil vs fish oil. *Wellness, nutrition.* 2016;365:1-2.
5. Schulze MB and Hu FB. Primary prevention of diabetes: What can be done and how much can be prevented? *Annual Review of Public Health.* 2005;26:445-67.
6. Borkman M, Storlien LH, Pan DA, Jenkins AB, Chisholm DJ and Campbell LV. The

- relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. *N Eng J Med.* 1993;328:238–44.
7. OECD. Guidance document on acute oral toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment. 2000;24.
 8. Kuhn L. The electrochemical society interface. *Winter.* 1998;5:26-31.
 9. Allain CC, Poon LS, Chan CS, Richmond W. and Fu PC. Enzymatic determination of total serum cholesterol. *Clin Chem.* 1974;20(4):470-5.
 10. Tiez NW. *A clinical guide to laboratory tests (2nd Ed)* Philadelphia: W.B. Saunders; 1990.
 11. Burstein M, Scholnick HR. and Morfin R. Rapid method for the isolation of lipoprotein from serum by precipitation with polyanions. *Scad J Clin Lab Inv.* 1970;40:583-95.
 12. Rauf A, Imran M, Abu-Izneid T, Ul-Haq I, Patel S, Pan X, Naz S, Silva AS, Saeed F and Suleria HAR. Proanthocyanidins: A comprehensive review. 2019;116(108999):1-6.
 13. Thakur M, Melzig FM and Weng A. Chemistry and pharmacology of saponins: special focus on cytotoxic properties: Review. *Bot: Tar and Ther.* 2011;1:19-29.
 14. Wang Q, Yang J, Zhang XM, Zhou L, Liao XL, Yang B. Practical synthesis of naringenin. *J Chem Res.* 2015;39:455–7.

© 2021 Orji et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/69795>