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Antioxidant Potentials of Blue Whiting Fish (*Micromesistus poutassous*) Oil Extracts in Streptozotocin Induced Diabetic Rats

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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.

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ABSTRACT

Aim: The aim of this study was to assess the antioxidant potentials of Blue Whiting Fish (*Micromesistus poutassous*) oil extracts in 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

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dose of Blue Whiting Fish (*Micromesistus poutassous*) oil extracts 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 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, total antioxidant capacity, malondialdehyde, superoxide dismutase and glutathione were analysed quantitatively and measured spectrophotometrically and the GC–MS analysis of bioactive compounds from Blue Whiting Fish (*Micromesistus poutassous*) 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: After week 1 - 4 of exposure, the mean SOD (Superoxide dismutase) value of the Negative control group (NC), Positive control (PC) group, diabetic groups exposed for weeks 1, 2, 3 and 4 expressed in U/ml were 299.41 ± 5.49, 217.38 ± 5.33, 220.56 ± 4.14, 240.45 ± 1.21, 258.19 ± 1.73 and 278.03 ± 1.98 respectively. The mean TAC (Total antioxidant capacity) value expressed in U/ml were 2.97 ± 0.10, 1.84 ± 0.04, 1.97 ± 0.04, 2.16 ± 0.02, 2.26 ± 0.02 and 2.46 ± 0.02 respectively. The mean GSH (Glutathione) expressed in µg/ml were 56.05 ± 0.60, 47.37 ±1.04, 47.94 ± 0.87, 50.80 ± 0.35, 53.07 ± 0.36 and 55.38 ± 0.33 respectively. The mean MDA (Malondialdehyde) expressed in nmol/l were 2.40 ± 0.13, 4.56 ± 0.28, 4.66 ± 0.06, 4.39 ± 0.01, 4.16 ± 0.03 and 3.70 ± 0.06 respectively. Comparison of different groups showed varying significant differences across groups.

Conclusion: Hyperglycaemia induced in rats studied led to an increase in oxidative stress, depletion of antioxidant parameters was observed. However, after treatment with Blue Whiting Fish (*Micromesistus poutassous*) Oil extracts, it was observed that there was improvement in the overall antioxidant status of the rats.

Keywords: Antioxidant; blue whiting fish (Micromesistus poutassous) oil extracts; streptozotocin induced diabetic rats.

1. INTRODUCTION

Diabetes mellitus which is among the non-communicable diseases is a major public health problem in most countries; Nigeria included [1]. The proportion of adult population with diabetes has been estimated and predicted to increase by 69% by the year 2030 [2]. In 2013, there were 382 million patients with diabetes worldwide, with an expected estimated rise to 592 million in 2035 [2].

Long term sustained hyperglycaemia induces excessive generation of reactive oxygen species leading to chronic oxidative stress [3], oxidative stress in turn causes damage to the beta cells by activating the stress induced pathway, and this invariably induces defective insulin biosynthesis and secretion and ultimately apoptosis. Insulin is insensitive in the midst of hyperlipidemia. Is there a link between insulin insensitivity and lipid metabolism? Considering the fact that diabetes leads to dyslipidemia that also causes hyperperoxidation which in turn releases free radicals into the system, there is need to develop a treatment regimen that will correct the dyslipidemia in diabetic complications as it has been reported that most disease conditions are propelled by depletion of antioxidants by free radicals. Omega 3 have been reported to have the potency of increasing the antioxidant capacity thereby slowing down the process of lipid peroxidation and all attendant complications arising from Type 2 DM.

Hyperglycaemia induces excessive generation of reactive oxygen species (ROS) which leads to chronic oxidative stress [4]. Clinical studies indicated that Diabetic patients are exposed to chronic oxidative stress and present increased pro-oxidants and markers of oxidative tissue damage [3]. Oxidative stress induced complications of diabetes may include stroke, neuropathy, retinopathy and nephropathy [5]. Therefore, the aim of this study was to assess the antioxidant potentials of Blue Whiting Fish (*Micromesistus poutassous*) oil extracts in 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 Rivers State University animal farm house is a research centre were consent has been given for the use of the laboratory animals for experimental study.

2.2 Treatments

Blue Whiting Fish (*Micromesistus poutassous*) Oil Extracts 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& 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 Blue Whiting Fish (*Micromesistus poutassous*) Oil Extracts and were sacrificed after one week.

Group 4: The animals in this group were induced with streptozotocin, became diabetic and were treated with Blue Whiting Fish (*Micromesistus poutassous*) Oil Extracts and were sacrificed after two weeks.

Group 5: The animals in this group were induced with streptozotocin, became diabetic and were treated with Blue Whiting Fish (*Micromesistus poutassous*) Oil Extracts and were sacrificed after three weeks.

Group 6: The animals in this group were induced with streptozotocin, became diabetic and were treated with Blue Whiting Fish (*Micromesistus poutassous*) Oil Extracts and were sacrificed after four weeks.

2.5 Blue Whiting Fish Oil (Kitchen Oven Extracted)

The Blue Whiting Fish (*Micromesistus poutassous*) oil (kitchen oven extracted) was subjected to proximate analysis and fatty acid profiling. The proximate analysis involves determination of moisture content, ash content, fat content, protein content, crude fibre and minerals.

2.5.1 Dose calculation of Blue Whiting Fish (*Micromesistus poutassous*) Oil (Kitchen oven Extracted)

The dose used was based on UNIH, 2016 which suggested that a total safe dose of 3 grams per day of combined DHA and EPA has been advised for adults by the FDA, with no more than 2 g from dietary supplements (UNIH, 2016).

Using the 3000mg daily safe dose for human.

Rat dose (mg/kg) =human dose x 0.018 x 5 [6].
=3000 x 0.018 x 5
=270mg/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 Total Antioxidant capacity (TAC), Superoxide dismutase (SOD), Malionaldehyde (MDA), Glutathione. The sample containers with anticoagulants were mixed thoroughly. Samples for fasting blood glucose were analysed within six (6) hours. 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 [7] 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 (H_2O_2). 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 Determination of Total antioxidant Capacity (TAC)

Total antioxidant capacity (TAC) was measured quantitatively by the ferric reducing antioxidant power (FRAP) method [8]. In an acidic environment, ferric – tripyridyltriazine ($Fe\ 3^{+}$-TPTZ) are reduced to the blue ferrous-tripyridyltriazine. The colour reaction reflects the total antioxidant capacity.

2.8.3 Determination of Superoxide dismutase (SOD) [9]

SOD level is measured quantitatively by measuring the activity of SOD. This method involves the enzymatic generation of the superoxide anion in the assay medium and competition between the superoxide scavenger and the SOD- catalyzed dismutation of superoxide. Superoxide dismutase (SOD, EC 1.15.1.1) is an enzyme that catalyzes the dismutation of the superoxide (O^{2-}) radical into either ordinary molecular oxygen (O^2) or hydrogen peroxide (H_2O_2). Superoxide is produced as a by-product of oxygen metabolism. The SOD enzymes deal with the superoxide radical by either adding or removing an electron from the superoxide molecules it encounters, thus changing the O^{2-} into one or two less damaging species: either molecular oxygen (O^2) or hydrogen peroxide (H^2O^2). The reduction reaction between O and reduced nitroblue tetrazolium (NBT) forms a blue colour, which has absorbance in 560nm; SOD reacts O^{2-} suppress the blue reaction. The deeper the blue colour, the lower the SOD activity.

2.8.4 Determination of Malondialdehyde [8]

MDA was estimated by a quantitative method. The principle of MDA is based on the quantification of a strong light absorbing and fluorescing adduct in a continuous reaction with thiobarbituric acid (TBA).

2.8.5 Determination of Glutathione (GSH) [10]

Glutathione reacts with 5,5’-dithiobis-2-nitrobenoic acid (DTNB) to form a yellow product which is read spectrophotometrically at 412nm.

2.8.6 Gas Chromatography-Mass Spectrometry (GC–MS) analysis

The GC–MS analysis of bioactive compounds from Blue Whiting Fish (*Micromesistus poutassous*) 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 × 250 μm in diameter × 0.25 μm 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.

2.8.6.1 Identification of chemical constituents

Bioactive compounds extracted from the Blue Whiting Fish (*Micromesistus poutassous*) oil

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. They were recorded as mean and standard deviations (Mean ± S. D), ANOVA (including Tukey's Multiple Comparison Test). Results were considered statistically significant at 95% confidence interval (*p < .05*).

3. RESULTS AND DISCUSSION

Bioactive compounds of the Blue Whiting Fish (*Micromesistus poutassous*) Oil Extracts used in this study indicate the presence of different types of phytochemicals in varying amounts (Tables 1 and 2a & 2b), example: Phenolic acids, alkaloids, flavonoids, saponins, Tannins in them In the treatment of Type 2 diabetes, heart disease, oxidative stress induced diseases 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 Blue Whiting Fish (*Micromesistus poutassous*) Oil Extracts for week one to four (Table 3). 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 Blue Whiting Fish (*Micromesistus poutassous*) Oil Extracts studied.

There is porosity of literature on the Blue Whiting Fish (*Micromesistus poutassous*) Oil Extracts used in this study. The results from this study showed significant difference (*P<.05*) in Superoxide dismutase (SOD) for rats supplemented with Blue Whiting Fish (*Micromesistus poutassous*) Oil Extracts. The SOD activity when compared with the Negative and positive controls, had a significant variation. In comparison with the positive control, there was a variation in SOD in an increasing order with an increase in duration at *P<.05* (Tables 3 and 4). The increase in the SOD compared to the positive control can be as a result of the reduction of oxidative stress in the diabetic animal which can be due to the presence of kaempferol, Naringenin [11].

Table 1. Quantitative and qualitative analyses of blue whiting fish (*Micromesistus poutassous*) oil extracts

Quantitative		Qualitative	
Phytochemicals	**Amount**	**Phytochemicals**	**Result**
Proanthocyanin ug/g	6.0193	Proanthocyanin ug/g	+ve
Oxalate ug/g	1.5784	Oxalate ug/g	+ve
Flavan 3 ol ug/g	-	Flavan 3 ol ug/g	-ve
Ribalinidine ug/g	4.1852	Ribalinidine ug/g	+ve
Naringenin ug/g	14.1512	Naringenin ug/g	+ve
Resveratol ug/g	-	Resveratol ug/g	-ve
Phenol ug/g	21.2507	Phenol ug/g	+ve
Epicatechin ug/g	15.6012	Epicatechin ug/g	+ve
Sapogenin ug/g	-	Sapogenin ug/g	-ve
Flavonones ug/g	14.4455	Flavonones ug/g	+ve
Phytate ug/g	4.3669	Phytate ug/g	+ve
Lunamarin ug/g	9.0116	Lunamarin ug/g	+ve
Kaempferol ug/g	15.0361	Kaempferol ug/g	+ve
Flavone ug/g	11.6931	Flavone ug/g	+ve
Quinine ug/g	-	Quinine ug/g	-ve
Anthocyanin ug/g	10.6842	Anthocyanin ug/g	+ve
Tannins ug/g	18.0204	Tannins ug/g	+ve
Catechin ug/g	41.2129	Catechin ug/g	+ve
Naringin ug/g	13.1109	Naringin ug/g	+ve
Rutin ug/g	5.4762	Rutin ug/g	+ve
Steroids ug/g	4.4192	Steroids ug/g	+ve

Table 2a. Blue Whiting Fish (*Micromesistus poutassous*) Oil Extracts on GC-MS

Chemical Name	Name	% comp
2-Fluorobenzyl		0.05
Cyclopropanemethanol	Organic compound	0.29
1-Nitro-2-acetamido-1,2-dideoxy-d-mannitol	Phytochemical	10.95
Butanoic acid	Organic acid	0.34
2H-Azepin-2-one	Phytochemical	9.31
2,4-Pentadien-1-ol	Aromatic compound	0.05
Undec-10-ynoic acid	Organic acid	0.28
Cyclopropanecarboxylic acid,	Organic compound	8.29
2-Propenoic acid	Organic acid	0.18
2,3-dihydroxypropyl ester	Essential oil	0.12
Undecylenic acid	Organic acid	0.31
Decosahexadecanoic acid	Fatty acid omega 3	3.16
d-Lyxose	Sugar	0.22
Cyclododecanol		9.09
4-Trifluoroacetoxytetradecane		0.04
Hexadecanoic acid	Fatty acid	0.10
9,12-octadecadienyloxy	Phytochemical	3.38
3-Dodecen-1-ol	Essential oil	1.97
-Heptadeca-1,9-dien-4,6-diyn3-ol	Phytochemical	0.05
9-Octadecenal	Essential oil	0.06
Cyclopropaneoctanal	Essential oil	1.93
11-Octadecenoic acid	Organic acid	8.01
Methyl stearate	Fatty acid	1.05
9,12-Octadecadienoic acid	Organic acid	0.31
cis-Vaccenic acid	Fatty acid	9.12
Oleic Acid	Fatty acid	2.42
Cycloeicosane		0.95
11-Dodecen-1-ol	Essential oil	0.37
9,12-Octadecadienoic acid	Fatty acid(omega 6	0.16
1,3,12-Nonadecatriene	Essential oil	0.03
2-hydroxy-1-(hydroxymethyl)ethyl ester	Phytochemical	0.06

Table 2b. Blue Whiting Fish (*Micromesistus poutassous*) Oil Extracts on GC-MS

Chemical Name	Name	% comp
2-Methyl-Z,Z-3,13-octadecadienol		8.08
9,17-Octadecadienal	Essential oil	0.16
Cyclopropaneoctanal	Essential oil	0.01
Hexadecyl propyl ether	Organic compound	0.08
Heptadecanolide	Organic acid	0.04
n-Propyl 11-octadecenoate	Phytochemical	0.65
2-Methyl-Z,Z-3,13-octadecadienol	Phytochemical	0.03
n-Hexadecanoic acid	Fatty acid	0.06
2-Methyl-Z,Z-3,13-octadecadienol	Phytochemical	3.05
Octadec-9-enoic acid	Fatty acid omega 3	0.11
6-Octadecenoic acid	Fatty acid	0.06
Ethyl Oleate	Fatty acid	7.09
1-Cyclohexylnonene	Phytochemical	0.07
2,3-Dihydroxypropyl elaidate	Phytochemical	0.01
1,2,3,6-tetrahydro-1-methyl-4-[4-chlorophenyl]	Phytochemical	0.07
1H-Indole-2-carboxylic acid	Phytochemical	0.22
tetrahydrofuran-2-yloxymethylene	Phytochemical	2.23
1,2-Benzenediol	Organic compound	0.13
Benzo[h]quinolone	Aromatic organic compound	0.07
4H-Pyran-3-carboxylic acid	Phytochemical	2.21
6-Chloro-1-ethyl-4-oxo-N-(pyridin--dihydro-4,4,6	Phytochemical	0.82
9,12,15-Octadecatrienoic acid	Fatty acid omega 3	1.10

Table 3. Mean antioxidant profile of control groups and group supplemented with Blue Whiting Fish (*Micromesistus poutassous*) Oil

Groups/Parmeters	SOD (U/ml)	TAC (U/ml)	GSH (µg/ml)	MDA (nmol/L)
Negative Control	299.41 ± 5.49	2.97 ± 0.1	56.05 ± 0.6	2.40 ± 0.13
DM Not Treated	217.38 ± 5.33	1.84 ± 0.04	47.37 ± 1.04	4.56 ± 0.28
DM + SO1	220.56 ± 4.14	1.97 ± 0.04	47.94 ± 0.87	4.66 ± 0.06
DM + SO2	240.45± 1.21	2.16 ± 0.02	50.80 ± 0.35	4.39 ± 0.01
DM + SO3	258.19 ± 1.73	2.26 ± 0.02	53.07 ± 0.36	4.16 ± 0.03
DM + SO4	278.03 ± 1.98	2.46 ± 0.02	55.38 ± 0.33	3.70 ± 0.06
p-value	0.0001	0.0001	0.0001	0.0001
F-values	72.44	62.98	29.99	37.96

Table 4. Post Analysis (Post Hoc) of antioxidant profile of control groups and group supplemented with Blue Whiting Fish (*Micromesistus poutassous*) Oil using the Tukey's multiple comparison test

Tukey's Multiple Comparison Test	SOD Summary	TAC Summary	GSH Summary	MDA Summary
Negative Control vs DM Group	***	***	***	***
Negative Control vs SO WK1	***	***	***	***
Negative Control vs SO WK2	***	***	***	***
Negative Control vs SO WK3	***	***	*	***
Negative Control vs SO WK4	**	***	NS	***
DM Group vs SO WK1	NS	NS	NS	NS
DM Group vs SO WK2	**	**	*	NS
DM Group vs SO WK3	***	***	***	NS
DM Group vs SO WK4	***	***	***	**
SO WK1 vs SO WK2	**	NS	*	NS
SO WK1 vs SO WK3	***	**	***	NS
SO WK1 vs SO WK4	***	***	***	***
SO WK2 vs SO WK3	*	NS	NS	NS
SO WK2 vs SO WK4	***	**	***	*
SO WK3 vs SO WK4	*	NS	NS	NS

*NS – Not significant; ; *, **, *** - Significant at p <0.05*

The results from this study showed significant difference (*P<.05*) in Total antioxidant capacity (TAC) for rats supplemented with Blue Whiting Fish (*Micromesistus poutassous*) Oil Extracts. The TAC activity when compared with the Negative and positive controls, had a significant variation. In comparison with the positive control, there was a variation in TAC in an increasing order with increase in duration at *P<.05* (Tables 3 and 4).

This significant increase in the TAC level is as a result of reduced ROS which in turn may result in reduced tissue damage by free radicals and can due to the presence of bioactive components such as catechins.

The results from this study showed significant difference (*P<.05*) in Glutathione (GSH) for rats supplemented with unprocessed fish oil. The GSH activity when compared with the Negative and positive controls, had a significant variation. In comparison with the positive control, there was a variation in GSH in an increasing order with an increase in duration at *P<.05* (Tables 3 and 4). The significant increase seen might suggest a possible effect of fish oil on antioxidant defence mechanisms of the cells which can be due to the presence of catechin, kaempferol, naringin.

The results from this study showed significant difference (*P<.05*) in Malonaldehyde (MDA) for rats supplemented with Blue Whiting Fish (*Micromesistus poutassous*) Oil Extracts (Tables 3 and 4). The MDA activity when compared with the Negative and positive controls, had a significant variation. In comparison with the positive control, there was a variation in MDA in a decreasing order with increase in duration at *P<.05*. This effect can be due to the presence of Naringenin which aids in lowering lipid peroxidation markers. [9]

4. CONCLUSION

Hyperglycaemia induced in rats studied led to an increase in oxidative stress, depletion of antioxidant parameters was observed. However,

after treatment with Blue Whiting Fish (*Micromesistus poutassous*) Oil Extracts it was observed that there was improvement in the overall antioxidant status of the rats.

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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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