IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 7, Issue 6 (Nov. – Dec. 2021), PP 50-56
www.iosrjournals.org

Oxidative Stress Status and Serum Lipid Profile of Wistar Rats Fed *Nigella sativa* Oil and *Canarium schweinfurthii* Oil Formulated Feeds

Ugorji, J.I., Onyewuchi, K. and Ezekwesili-Ofili, J.O
Applied Biochemistry Department, Nnamdi Azikiwe University, Awka, Nigeria.

Abstract: *This study was carried out to investigate the oxidative stress status and serum lipid profile of wistar rats fed Nigella sativa oil and Canarium schweinfurthii oil formulated feeds. A total of 25 albino rats of wistar strain were used for the animal study. The rats were divided into four groups (n=5) according to the diets given to them which consisted of a normal basal diet group (negative control), 10% of olive oil formulated feed group (standard diet), 10% of Nigella sativa oil formulated feed group (NSO), and 10% of Canarium schweinfurthii oil formulated feed group (CSO) for an experimental feeding period of 21days. Blood samples were collected via cardiac puncture and serum prepared for biochemical analysis. The results obtained showed that all experimental groups had significant increase (P<0.05) between their mean initial and final weights. There was no significant difference (P>0.05) in the malondialdehyde levels; a marker for oxidative stress, of the experimental groups. It was observed that there was no significant difference (P>0.05) in the superoxide dismutase activity, while there was a significant difference (P<0.05) in the catalase activity. The significantly increased catalase activity of the NSO group which is 0.017±0.001u/l indicated improved antioxidant status. Also, there was no significant difference (P>0.05) in the total cholesterol, triglycerides, high density lipoproteins (HDL)-cholesterol and low density lipoproteins (LDL)-cholesterol levels of all the experimental groups. Notably, it was observed that there was a decrease total cholesterol levels in both the NSO* (111.80±15.03 mg/dl) *and CSO* (149.70±21.40 mg/dl) *group, while the NSO group had the lowest LDL levels of 86.15±16.44 mg/dl. This study demonstrated that Nigella sativa oil and Canarium schweinfurthii oil are nutritionally acceptable and suitable edible vegetable oils on cardiovascular health.*

Keywords*: Nigella sativa oil, Canarium schweinfurthii oil, superoxide dismutase, catalase, MDA, oxidative stress and serum lipid profile.*

Date of Submission: 15-12-2021 Date of Acceptance: 31-12-2021

I. Introduction

Edible vegetable oils are triglycerides of plant origin that include olive, palm, soybean, canola, and sunflower oil. Various seeds, fruits, kernels, and nuts are used as sources in the production of edible oils. They are important nutritional components with variety of functions in the body as an energy source, component of membrane structures, for body temperature regulation and organs insulation (Negash *et al*, 2019).

The quality of dietary oils depends on its level of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and especially on the ratio of omega-6 to omega-3 fatty acids (Vetrani, 2012). Saturated fatty acids (SFA), which raise cholesterol levels, are promoters of coronary heart disease, whereas the omega-3 and omega-6 isomers of PUFA, MUFA, and antioxidants have been described as protecting against coronary heart disease (Schwab *et al*, 2014). However, it is not only the composition of fatty acids that affects the nutritional quality of fat. The efficacy of α-tocopherol has been shown in the prevention and treatment of heart disease, cancer, and Alzheimer's disease (Tucker and Townsend, 2005). For this reason, plant oils are a good source of compounds that can decrease the risk of some diseases and growing consumer awareness has led to more interest in natural plant oils, which are often considered functional foods (Qian *et al*, 2018).

Nigella sativa (NS), or also known as black cumin or its Arabic name *habat-ulsauda*, has been used for centuries in medicinal and culinary purposes throughout the Middle East, India, and Northern Africa. It is an annual flowering plant with pale blue flowers that belongs to the *Ranunculaceae* family and grows in Southwest Asia, the Middle East, Southern Europe, and Northern Africa (Ahmad *et al*, 2013). The plant has a fruit which contains angular black seeds, and the seeds are considered to be the most valuable part contributing beneficial health effects (Xin-Fang *et al*, 2013).

Commonly referred to as simply black seed, *N. sativa* seeds go by many other names, including black cumin, black caraway, nigella, kalonji, fennel flower, and Roman coriander. Black seed oil obtained from

N.sativa seeds have been used in traditional medicine for over 2000 years due to its many therapeutic benefits (Tavakkoli *et al*, 2017).

Studies suggest that black seed oil may have numerous applications for health, including the treatment of asthma and aiding weight loss. It is also applied topically to benefit skin and hair (Kehhanmanesh *et al*, 2014; Padhye *et al*, 2008). The seeds and oils of *N. sativa* are also good food components to be used in the dietary systems as a spice and food preservative. Besides, both seeds and oils have been reported to have several biological activities including antioxidant, antimicrobial, antihypertensive, anticancer, anti-inflammatory, diuretics, anti-diarrheal, appetite stimulant, and analgesics, therefore they can be considered as functional food components (Rohman *et al,* 2019).

Canarium schweinfurthii belongs to the *Burseraceae* family and grows in the equatorial forest region and is widely distributed in tropical Africa, particularly across East, Central and West Africa, so it is usually referred to as African olive or black olive (Mogana and Wiart, 2011; Keary, 1989). It thrives well in the rocky and flat lands of Plateau state of Nigeria. This plant has different names depending on the language and country where it grows: English (purple canary tree, incense tree, gum resin tree, bush candle tree, African elemi); French (elemier d'Afrique, elemi de Moahum, elemi d'ouganda); Luganda (muwafu); Swahili (mpafu, mbani); Beh (in West Cameroon); Igbo (Ube okpoko); Hausa (Atili) (Ngbebe *et al*, 2008).

The plant produces oblong fruits of different sizes ranging from 1.84-3.06 cm long and has a sharp point at the apex and a persistent calyx at the base. The fruits are similar in structure and colour to the well-known fruits of olive (*Olea europaea*), though from different families (Nyam *et al*, 2014).The fruit pulp (mesocarp) is commonly eaten raw or cooked and it is also usually processed for the constituent oil which is popularly referred to as Atili oil in some parts of Northern Nigeria (Olawale, 2012). Atili oil has a nutty and pleasant smell, and has been used in preparing meals. It is believed to contain vitamin E that nourishes the skin and stimulate hair growth and has served as carrier oil in skin and hair care products. The fruits have served humans for centuries as snack and oil from the fruits have served humans for domestic, pharmaceutical and industrial purposes (Nyam *et al*, 2014).

Diet and some of its components could influence the intensity of oxidative stress (Vetrani *et al*, 2012). Oxidative stress resulting in increased lipid peroxidation and cholesterol levels has been linked to the etiology of various pathological states like inflammation, atherosclerotic cardiovascular disease and coronary heart disease. Although reactive oxygen species produced as natural by-products during normal metabolism have important roles in cell signalling and homeostasis, these reactive oxygen species can initiate lipid peroxidation. The increased lipid peroxidation leads to the oxidative modification of low-density lipoprotein (LDL-cholesterol), which plays a key role in the pathogenesis of atherosclerosis. Apart from this, reactive oxygen species can also damage other biological macromolecules like protein and DNA; these biological effects mediated by the reactive oxygen species are controlled by various endogenous defence mechanisms consisting of enzymatic and non-enzymatic scavenger components (Arunima and Rajamohan, 2013).

Vegetable oil is one of the essential dietary components in daily food consumption and the quality of dietary oils has been widely recognized to be inextricably linked to the pathogenesis of cardiovascular disease which is one of the leading major causes of morbidity and mortality worldwide. Cardiovascular diseases are a substantial and growing problem in most of the developing regions of the world and continue to remain a growing health concern worldwide.

Nigella sativa oil and *Canarium schweinfurthii* oil has been used for various domestic, medicinal and industrial purposes, but there has been limited dietary research on them and evaluating the cardiovascular health benefits and safety in the consumption of these vegetable oils, is a matter of significant interest to consumers. Hence, this study is designed to evaluate the oxidative stress status and serum lipid profile of animals fed *Nigella sativa* oil and *Canarium schweinfurthii* oil formulated feeds.

II. Materials and Methods

Oils

Nigella sativa (black seed) oil, and *Canarium schweinfurthii* (Atili) oil were obtained from retail distributors in Anambra, Nigeria.

Chemicals/Reagents

All chemicals/reagents used in this work were of analytical grade.

Diet Formulation

Feeds were formulated by mixing 10% of *Nigella sativa* oil (NSO), *Canarium schweinfurthii* oil (CSO) and olive oil respectively, with a basal rat diet comprising of 65% of corn flour, 22% of crayfish, and 3% of premix (weight/weight %). The formulated feeds were pelletized and stored in a cool dry environment.

Experimental Animals

Twenty-five male wistar albino rats weighing between 80-100g were obtained from the animal breeding house in Awka. The rats were kept in well aerated cages and allowed to acclimatize for seven days before the commencement of the experimental feeding period. They were maintained under ambient conditions, with normal rat diet and water provided *ad libitum*. The animals were handled in accordance with the guidelines of the Ethics Committee on Animal Research of the Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria.

Experimental Design

After acclimatization, baseline samples were collected before the experimental feeding period for biochemical analysis. The rats were randomly divided into four groups (n=5) based on their experimental diets:

- **Group 1**(control) was given basal diet; (negative control).
- **Group 2** was given olive oil formulated feed; (standard diet).
- **Group 3** (NSO) was given *Canarium schweinfurthii* oil formulated feed.
- **Group 4** (CSO) was given *Nigella sativa* oil formulated feed.

The rats were allowed free access to feed and drinking water *ad libitum* for 21 days. The body weights of the animals were also recorded at weekly intervals using a digital weighing balance.

Blood Collection and Preparation of Serum

At the end of the experimental feeding period, the rats were anaesthetized using chloroform after an overnight fast. Blood samples were then collected via cardiac puncture using 10ml syringes and placed into plain sampling bottles. The sera samples were obtained as the supernatant after centrifuging the coagulated blood samples at 4,000 r.p.m for 10 minutes and then labelled.

Assay of Malondialdehyde (MDA) levels

Malondialdehyde (MDA), an index of lipid peroxidation, was determined using the method of Buege and Aust (1978).

Determination of Superoxide Dismutase (SOD) activity

Superoxide dismutase activity was determined as described by Sun and Zigma, (1978).

Catalase (CAT) activity

Catalase activity was determined according to the method of Sinha (1972).

Determination of Serum Lipid Profile

Total cholesterol, Triglycerides, Low-Density Lipoprotein (LDL) and High-Density Lipoprotein (HDL) were determined according to the method of Sidhu and Naugler (2012). The Friedewald's method was used for calculating Very Low-Density Lipoproteins (Friedewald *et al*, 1972).

Statistical Analysis

Result of biochemical parameters were expressed as mean ± SEM. Significant difference between mean groups was determined by one way analysis (ANOVA). P value of < 0.05 was considered significant.

III Results

The results of the percentage change in weight, MDA levels, catalase activity, superoxide dismutase activity and serum lipid profile of the experimental animals are shown in Fig. 1,2,3,4, and Table 1 respectively.

Percentage change in weight of the animals:

The results of the average weight of the animals showed that all the animals in all the groups gained weight between the initial and final day of the animal study.

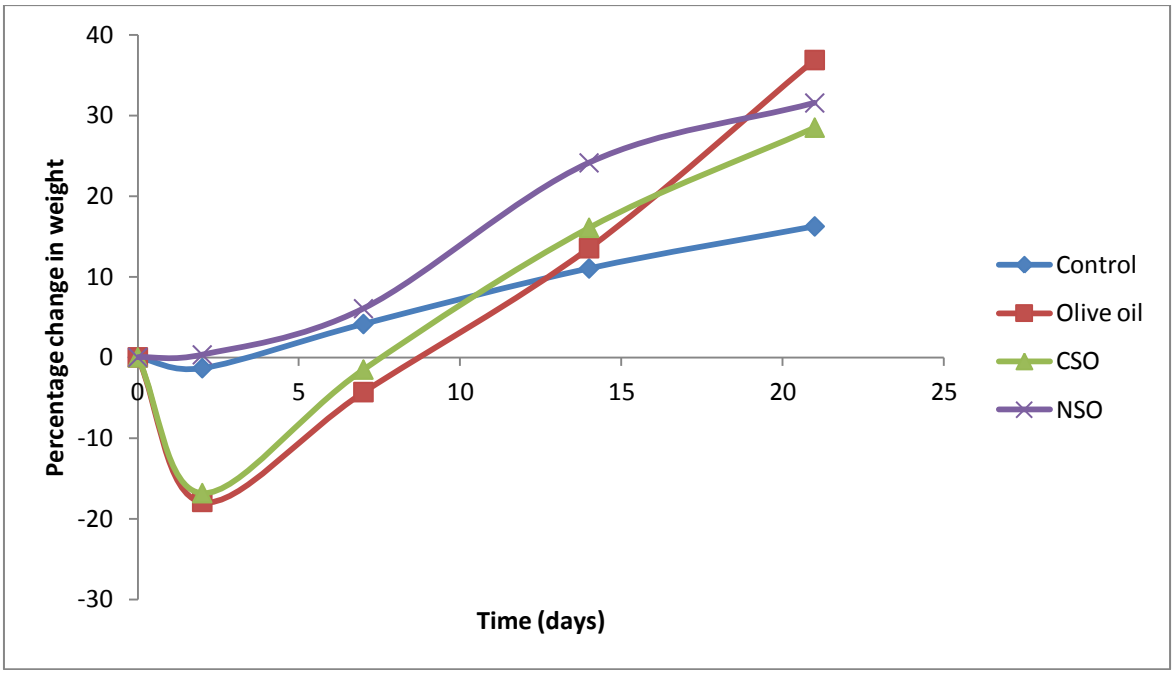

Fig 1: Percentage change in weight (g) of the experimental groups with time.

Malondialdehyde (MDA) levels

The NSO group had the lowest average MDA levels while the CSO group had the highest average MDA levels. There was no significant difference (P>0.05) observed in the MDA levels.

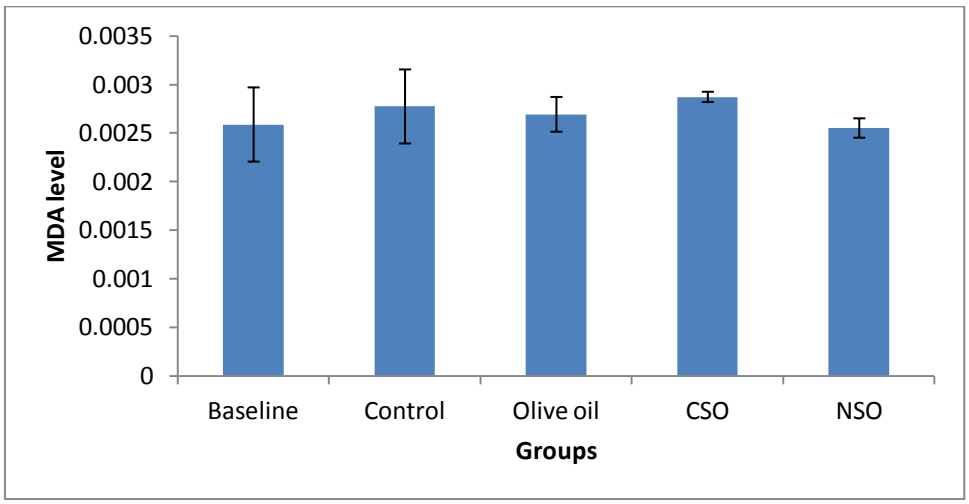

Fig 2: MDA levels (µm/l) of the experimental animals.

Catalase Activity

There was increase in the catalase activity of the experimental oil groups when compared to the baseline and control. The NSO group had the highest catalase activity which was significantly higher (P<0.05) than the olive oil and CSO group.

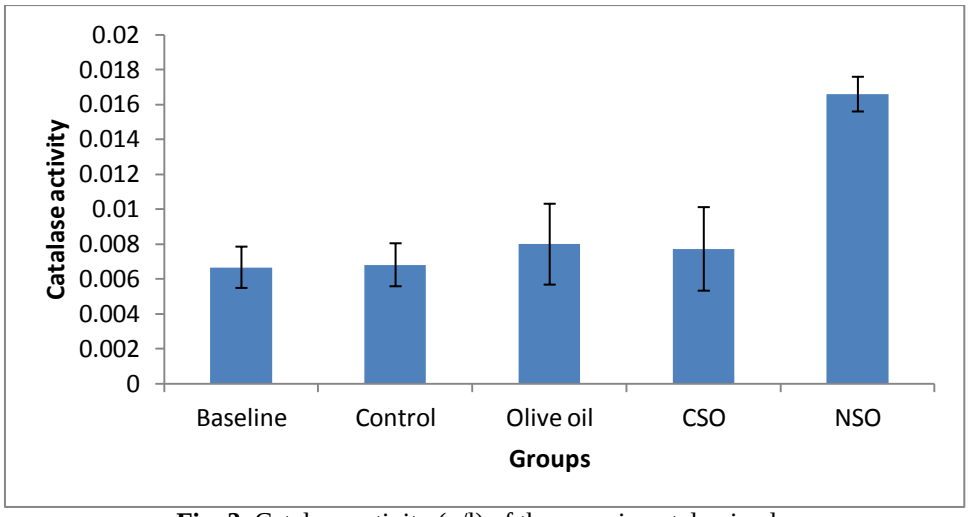

Fig. 3: Catalase activity (u/l) of the experimental animals.

Superoxide dismutase (SOD) Activity

The CSO group had the highest superoxide dismutase activity while the NSO group had the lowest superoxide dismutase activity. There was no significant difference (P>0.05) in SOD activity.

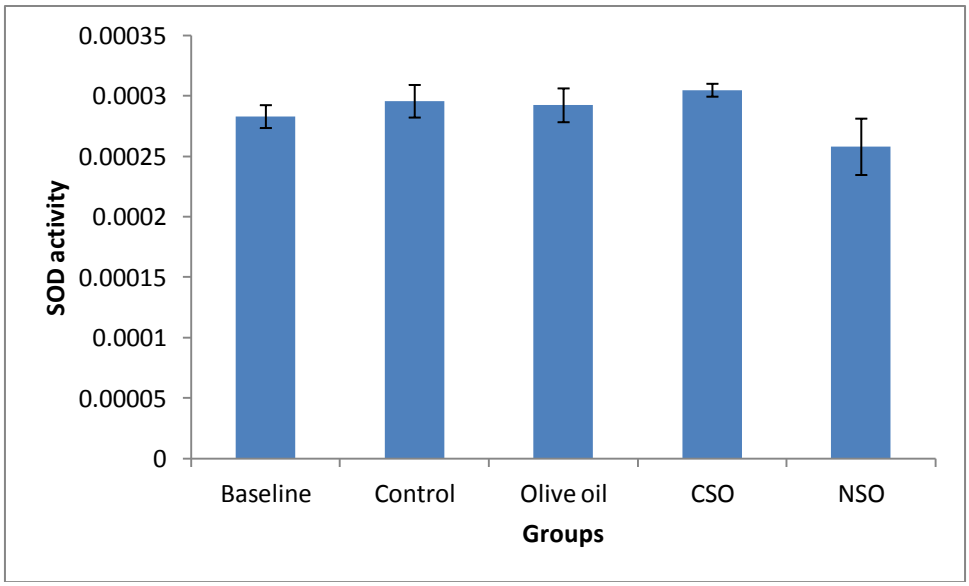

Fig. 4: SOD activity (u/l) of the experimental animals.

Serum Lipid Profile Levels

The CSO group and NSO group had decreased total cholesterol levels compared to the control group. The olive oil group had the highest total cholesterol levels. There was no significant difference (P>0.05) in the total cholesterol levels.

The NSO group had the lowest triglyceride levels while the CSO group had the highest triglyceride levels. There was no significant difference (P>0.05) in the triglyceride levels.

The olive oil group, CSO group and NSO group had decreased HDL levels compared to the control. CSO group had the lowest HDL levels. There was no significant difference (P>0.05) in the HDL levels.

The olive oil group and CSO group had increased LDL levels while NSO group had decreased LDL levels compared to the control. There was no significant difference (P>0.05) in LDL levels.

The NSO group had the lowest VLDL levels while the CSO group had the highest VLDL levels.

Table 1: Serum Lipid Profile levels of the experimental animals

Groups	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Baseline	173.28±20.19	121.38±13.64	47.32±12.93	104.25±23.09	24.28±2.73
Control	169.67±14.46	116.91±10.31	53.18±9.04	101.95±23.48	23.38±2.06
Olive oil	182.69±24.56	117.52±22.27	50.41±13.05	108.78±15.93	23.50±4.45

CSO	149.70±21.40	146.24±6.99	39.92±9.44	107.20±18.58	29.25±1.40
NSO	111.80±15.03	100.66±24.54	49.14±15.71	86.15±16.44	20.13±4.91

HDL; High-Density lipoproteins; LDL: Low-Density lipoproteins; VLDL: Vey Low-Density lipoproteins

III. Discussion and Conclusion

Discussion

Evidence from experimental, clinical and epidemiological studies has unequivocally pointed to oxidative stress as the key culprit in the pathogenesis of cardiovascular diseases (Ceconi *et al,* 2003; Dalle-Donne *et al,* 2006). Oxidative stress acts mainly as a trigger of atherosclerosis. It is well known that atheromatous plaque formation results from an early endothelial inflammation, which in turn leads to reactive oxygen species (ROS) generation by macrophages recruited *in situ.* The circulating low density lipoproteins (LDL-cholesterol) are then oxidized by reactive oxygen species, thus leading to foam cell formation and lipid accumulation. The result of these events is the formation of an atherosclerotic plaque. Both *in vivo* and *ex vivo* studies provided evidences supporting the role of oxidative stress in atherosclerosis, ischemia, hypertension, cardiomyopathy, cardiac hypertrophy, and congestive heart failure (Bahorun *et al,* 2006; Droge, 2002; Chatterjee *et al,* 2007; Ceriello, 2008).

From the animal study, the results obtained showed that all experimental groups had significant increase ($P<0.05$) between their mean initial and final weights.

There was no significant difference ($P>0.05$) in the malondialdehyde (MDA) levels, which is an index of lipid peroxidation, in the experimental groups.

Lipid peroxidation of polyunsaturated lipids is one of the most preferred markers for oxidative stress. The product of lipid peroxidation, malondialdehyde, is easily detected in blood and has been used as a measure of oxidative stress. The peroxidized lipid can produce peroxy radicals and singlet oxygen. In addition, the unsaturated aldehydes produced from these reactions have been implicated in modification of cellular proteins and other constituents that could determine a loss, or impairment of their enzymatic activity (Halliwell, 2007; Frei, 1997; Marnett, 2000; Gabriele *et al,* 2017).

The human body put in place several strategies to counteract the effects of free radicals and oxidative stress, based on enzymatic (e.g., SOD, CAT, and GPx) and non-enzymatic (e.g., lipoic acid, glutathione, L-arginine, and coenzyme Q10) antioxidant molecules, all of them being endogenous antioxidants.

From the study, there was no significant difference ($P>0.05$) in the superoxide dismutase activity while there was a significant increase ($P<0.05$) in the catalase activity of the NSO group. The catalase activity of the NSO group, which was 0.017±0.001u/l was significantly higher ($P<0.05$) when compared to the baseline (0.007±0.001u/l), control (0.007±0.001u/l), CSO (0.008±0.002u/l) and olive oil (0.008±0.002u/l) group.

Antioxidant enzymes such as catalase and superoxide dismutase mutually act against free radical mediated damage and prevent lipid peroxidation (Arunima and Rajamohan, 2016). Increased activity of catalase in the NSO group showed improved antioxidant status which could be a compensatory regulatory response to increased free radical generation.

There was no significant difference ($P>0.05$) in the serum lipid profile levels (total cholesterol, triglycerides, HDL, LDL) in all the experimental groups. There was a non-significant decrease in the total cholesterol levels of the CSO (149.70±21.40 mg/dl) and NSO (111.80±15.03 mg/dl) group.

Notably, it was observed that the NSO group had the lowest LDL levels of 86.15±16.44 mg/dl, which was a decrease in LDL levels compared to the baseline (104.25±23.09 mg/dl), CSO group (107.20±18.58 mg/dl), olive oil group (108.78±15.93 mg/dl) and control group (101.95±23.48 mg/dl).

This agrees with Sahebkar *et al,* (2016) that reported that the black seed oil reduced the total cholesterol (TC), low–density lipoprotein–C (LDL–C), thyroglobulin (TG) with an increased high–density lipoprotein–C (HDL–C) level.

IV. Conclusion

This study showed that there was no significant alteration in the lipid peroxidation index, malondialdehyde; an oxidative stress marker, and serum lipid profile of rats investigated among the different experimental groups. It is worthy of note that data from this study demonstrated that *Nigella sativa* oil and *Canarium schweinfurthii* oil are nutritionally acceptable and suitable edible vegetable oils on cardiovascular health. The decreased total cholesterol levels of the NSO and CSO group suggests *Nigella sativa* oil and *Canarium schweinfurthii* oil to be heart-friendly in reducing the risk of cardiovascular diseases. The increased catalase activity of the NSO group suggests free radical scavenging activity of *Nigella sativa* oil. Since vegetable oils are usually part of our staple diets, further research on the consumption of these oils needs to be carried out to determine their long term health safety and benefits.

References

[1]. Ahmad, A., Husain, A., Mujeeb, M., Khan, A.K., Najmi, A.K., Siddique, N.A., Damanhouri, Z.A., and Anwar, F. (2013). A review on therapeutic potential of *Nigella sativa*: A miracle herb. *Asian Pacific Journal of Tropical Biomedicine*. 3(5): 337-352. doi: 10.1016/S2221-1691(13)60075-1.

[2]. American Public Health Association (APHA) (2005). Direct Air- Acetylene Flame Method. *Standard Methods for the examination of metals*. 21st Edition.

[3]. Arunima, S., and Rajamohan, T. (2013). Effect of virgin coconut oil enriched diet on the antioxidant status and paraoxonase 1 activity in ameliorating the oxidative stress in rats – a comparative study. *Food and Function*. 4:1402. DOI: 10.1039/c3fo60085h.

[4]. Friedewald, W.T., Levy, R.I., Fredrickson, D.S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*. 18(6): 499-502.

[5]. Keary, R.W.J. (1989). A revised version of Nigeria trees. *Lavendon Press, Oxford, England*. 476.

[6]. Keyhanmanesh, R., Gholamnezhad, Z., and Boskabady, M.H. (2014). The relevant effect of *Nigella sativa*on smooth muscles, its possible mechanisms and clinical applications. Iranian Journal of Basic Medical Sciences. 17(12): 939-949.

[7]. Mogana, R., Wiart C. (2011). Canarium L: A Phytochemical and Pharmacological Review. *Journal of Pharmarcy Research*. 4(8):2482-89.

[8]. Mogana, R., Wiart C. (2011). Canarium L: A Phytochemical and Pharmacological Review. *Journal of Pharmarcy Research*. 4(8):2482-89.

[9]. Negash, Y.A.,Amare, D.E., Bitew, B.D., and Dagne, H. (2019). Assessment of quality of edible vegetable oils accessed in Gondar City, Northwest Ethiopia. *BMC Research Notes*.12:793. https://doi.org/10.1186/s13104-019-4831-x

[10]. Ngbebe, J., Yakubu, R.A., Nyam, D.A. (2008). Phytochemical screening for active compounds in Canarium schweinfurthii (Atile) Leaves from Jos North, Plateau State Nigeria. *Research Journal of Biological Sciences*. 3(9):1076-8.

[11]. Nyam, M.A., Makut, M.D., Itelima, J.U., and Daniel, A.M. (2014). Nutritional Potential of the Fruits of Black Olive (Canarium schweinfurthii Linn) from Plateau State, Nigeria. *Pakistan Journal of Nutrition*. 13 (6): 335-339.

[12]. Olawale, A.S. (2012). Solid-liquid extraction of oils of African elemi's (Canarium schweinfurthii's) fruit. *Agricultural Engineering International: CIGR Journal*. 14(2): 155-161.

[13]. Padhye, S., Banerjee, S., Ahmad, A., Mohammad, R., and Sarkar, F.H. (2008). From here to eternity – the secret of Pharaohs: Therapeutic potential of black cumin seeds and beyond. Cancer therapy. 6(b): 495-510

[14]. Qian, Y., Paulina, W., Aleksander, S., Anna, K., and Magdalena, R. (2018). Phytochemical Content, Oxidative Stability, and Nutritional Properties of Unconventional Cold-pressed Edible Oils. *Journal of Food and Nutrition Research*. 6(7): 476-485. DOI: 10.12691/jfnr-6-7-9.

[15]. Rohman, A., Lukitaningsih, E., Rafi, M., Nurrulhidayah, A.F. and Windarsih, A. (2019). *Nigella sativa* oil: physico-chemical properties, authentication analysis and its antioxidant activity. *Food Research*. 3(6): 628 – 634

[16]. Schwab, U., Lauritzen, L., Tholstrup, T., Haldorsson, T.I., Riserus, U. and Becker, W. (2014). Effect of the amount and type of dietary fat on cardiometabolic risk factors and risk of developing type 2 diabetes, cardiovascular diseases, and cancer: A systematic review. *Food and Nutrition Research*. 58: (25)145.

[17]. Sidhu, D., and Naugler, C. (2012). Fasting time and lipid levels in a community-based population: a cross-sectional study. *Archives of Internal Medicine*. 172(22): 1707-10. Doi: 10.1001/archinternmed.2012.3708. PMID:23147400.

[18]. Sinha, K.A. (1972). Colorimetric Assay of Catalase. *Analytical Biochemistry*. 47:389-394.

[19]. Sun, M., and Zigma, S. (1978). An improved Spectrophotometer Assay of Superoxide Dismutase Based on Epinephrine Antioxidation. *Analytic Biochemistry*. 90: 81-89.

[20]. Tavakkoli, A., Mahdian, V., Razavi, B.M., and Hosseinzadeh, H. (2017). Review on Clinical trials of Black Seed (Nigella sativa) and Its Active Constituent, Thymoquinone. *Journal of Pharmacopuncture*. 20(3): 179-193. doi: 10.3831/KPI.2017.20.021

[21]. Tucker, J.M., and Townsend, D.M. (2005). Alpha-tocopherol: Roles in prevention and therapy of human disease. *Biomedicine and Pharmacotherapy*. 59:380-387.

[22]. Vetrani, C., Costabile, G., Di Marino, L., and Rivellese, A.A. (2012). Nutrition and oxidative stress: a systematic review of human studies. *International Journal of Food Sciences and Nutrition*. 1-15.

Ugorji, J.I, et. al. "Oxidative Stress Status and Serum Lipid Profile of Wistar Rats Fed Nigella sativa Oil and Canarium schweinfurthii Oil Formulated Feeds." *IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)*, 7(6), (2021): pp. 50-56.