

Inflammatory and Lipid Peroxidation effects of Canola Oil, Extra Virgin Olive Oil, and Sunflower Oil on Albino Rats Fed With the Oils

Okafor, C.S^{1*}, Ogbachebe, C.C¹, Okwubanego, M.K¹, Enemor, V.A¹,
Eneh. F.U¹ and Nwenyi. V².

¹Applied Biochemistry Dept, Nnamdi Azikiwe University, Awka, Nigeria.

Abstract: Several vegetable oils have been associated with beneficial or harmful effects on the cardiovascular system which arises from the level of saturation or unsaturation of such oils. A total of 20 rats were used for the experiment for a period of 21 days. The rats were grouped into four according to the feed given to them which consisted of a normal rat feed control group (group A), formulated ration of rat feed and extra virgin olive oil group (group B), formulated ration of rat feed and canola oil group (group C), and formulated ration of rat feed and sunflower oil group (group D), all in a ratio of 4(feed):1(oil). At the end of the experiment, the blood was collected by cardiac puncture. The blood was collected in both plain bottles and EDTA anti-coagulant bottles for analysis. The result showed that except for group B, all the groups showed significant increase ($P < 0.05$) between their mean initial and final weights. The catalase result for olive oil, canola oil and sunflower oil were 0.293 ± 0.075 IU, 0.429 ± 0.147 IU and 0.527 ± 0.022 IU respectively, but were not significantly different ($P > 0.05$) when compared to the catalase level in the control group. The superoxide dismutase activity (SOD) of rats fed with canola oil, sunflower oil as well as the control group were 0.037 ± 0.022 IU, 0.043 ± 0.021 IU and 0.014 ± 0.002 IU respectively which were higher than those fed with olive oil (0.012 ± 0.007 IU), but there was also no significant difference when the superoxide dismutase activity was compared in all the rat groups. There was no significant difference ($P > 0.05$) in both Erythrocyte Sedimentation Rate (ESR) and Malondialdehyde Activity (MDA) levels in all the groups. Group C had the highest MDA levels of 0.324 ± 0.085 μ M which was higher than that of the control (group A) which had MDA levels of 0.288 ± 0.053 μ M; Group B had the lowest MDA levels of 0.188 ± 0.043 μ M; Group D had the highest ESR levels of 3.667 ± 0.667 mm/hr which was above that of the control which had ESR levels of 2.000 ± 0.577 mm/hr; Group B had the lowest ESR levels of 1.667 ± 0.333 mm/hr. This suggests that although there was no significant difference in the lipid peroxidation and inflammatory parameters investigated there are still some concerns about the type of oils that we consume which can predispose our systems to lipid peroxidation and inflammation. Monounsaturated oil (olive oil) seemed to be better in terms of the parameters investigated.

Keywords: Sunflower oil, canola oil, extra virgin olive oil, Catalase, MDA, ESR, and SOD.

Date of Submission: 21-05-2020

Date of Acceptance: 08-06-2020

I. Introduction

Vegetable oil is very common, affordable and used by majority of the people across the globe especially in the tropics. Its use as an antidote to prevent some oxidative stress related disease and complication has been advocated (Oguntibeju et al., 2010). Vegetable oils in particular are natural products of plant origin consisting of ester mixtures derived from glycerol with chains of fatty acids containing about 14 to 20 carbon atoms with different degrees of unsaturation (Emmanuel and Mudiakoghene, 2008). The proper amount and form of vegetable oil with an optimal ratio of omega 3 to omega 6 fatty acids are essential aspect of a well balanced diet and factors for the sufficient development of an organism. However, consumption of excessive amount of fats (even vegetable oil) in their non-oxidized or oxidized form in a high-fat diet may be an oxidative stress inductor, which in turn may damage proteins, lipids and carbohydrate and causes many diseases (Attori et al., 2010). Among the edible vegetable oils that have drawn considerable interest for both their antioxidant and health promoting effects include extra virgin olive oil which contains polyphenols as part of its antioxidant component, sunflower oil, which contains phenolic compounds as its main antioxidant compound and canola oil which 2,6-dimethoxy-4-vinylphenol also known as canolol as the most active antioxidant component in the oil.

Olive oil is a key component of the Mediterranean diet and recognized to contribute to its health benefits, especially in preventing cardiovascular diseases (CVD) (Lopez-Miranda et al., 2010). Recent trials on olive oil supplementation point towards a protective effect against cardio metabolic risk factors. The dietary benefits of olive oil were initially linked to its high oleic acid content, a monounsaturated fatty acid (18:1 n-9),

which ranges from 55% to 83% of total fatty acids. However, experimental-based evidence has accumulated on the health benefits of minor bioactive components of olive oil which have specific structures and are found in high quantities in virgin and extra-virgin olive oil. Among these structures, hydroxytyrosol was recently reported to protect the blood lipids against oxidative damage as reported by the European food safety authority (EFSA) panel on dietetic products, nutrition and allergies. The Panel considered that in order for olive oil to bear the “heart-health” claim, 5 mg of hydroxytyrosol and its derivatives (e.g. oleuropein complex and tyrosol) in olive oil should be consumed daily (Martin-Pelaez *et al.*, 2013).

Sunflower oil is an excellent source of vitamin E /tocopherol which neutralizes free radicals, scavenge them and prevent oxidative damage to cellular and molecular components exhibiting anti-inflammatory, cardio protective and anti tumour action. Due to anti-inflammatory action of tocopherols, sunflower seeds seem to have a promising role to play in chronic inflammatory conditions like bronchial asthma, osteoarthritis and rheumatoid arthritis. Also important impact of vitamin E on cardiovascular system makes sunflower seed oil beneficial in reducing atherosclerosis and hence complications like coronary artery disease and stroke (Ukiya *et al.*, 2007). Sunflower oil is also high in oleic acid (C18:1) and linoleic acid (C18:1). Sunflower seeds possess antioxidant value 0.153, antioxidant activity 72.9, oxidation rate ratio 0.271 and antioxidant activity coefficient 279.7 which has no statistically significant correlation with total phenolic extracts (Eichenfield *et al.*, 2009).

Canola oil is characterized by the following: low level (7%) of saturated fatty acids (SFAs); substantial amounts of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), including 61% oleic acid, 21% linoleic acid, and 11% alpha-linolenic acid (ALA) (Johnson *et al.*, 2009); plant sterols (0.53–0.97%); and tocopherols (700–1,200 ppm) – all of which have data indicating they are cardioprotective substances. With regard to the high MUFA content of canola oil, Hunter *et al.* (2010) and Gillingham *et al.* (2011) have provided evidence supporting positive effects of MUFAs compared with SFAs on cardiovascular health through the regulation of plasma lipids and lipoproteins, susceptibility of low-density lipoprotein. Evidence from experimental, clinical and epidemiological studies has unequivocally pointed out lipid peroxidation as the key marker of inflammation, cardiovascular disease as well as some forms of cancer.

CVD continues to remain a concern in developed countries and is a growing health concern worldwide. Although death rates from CVDs have decreased in many countries due to advances in medicine, the prevalence of CVD risk factors continues to increase. Due to the lifetime consumption of vegetable oils by humans, this study aimed to investigate the levels of some serum inflammatory markers such as MDA and ESR, and antioxidant enzymes present in some wistar albino rats fed with vegetable oil formulated diets.

II. Materials and Methods

Source of sample

The vegetable oils (Canola oil and Sunflower oil) used for this work were bought from Onitsha main market, Anambra State, Nigeria. The Extra virgin Olive oil was bought from the Holy Trinity Basilica, Onitsha, Anambra state, Nigeria.

Animals

The animals (male Wistar Albino rats, 20 in number) were gotten from a private farm at Ifite Awka, Awka North L. G. A., Anambra State, Nigeria. They were acclimatized in four standard cages for one week. Each cage contained five rats each. The animals were housed in the Applied Biochemistry Department animal house, Nnamdi Azikiwe University, Awka, Nigeria. Feed and water were provided for them *ad libitum*.

Diet formulation

Normal commercial rat feed was used in diet formulation. It contained all the nutrients required by the body. Crushed normal commercial rat feed was blended with the various vegetable oil samples in the ratio of 4:1 (wt/wt) respectively. The formulations were re-pelletized and stored in a cool dry environment. Attempts were made to avoid exposure of the formulated feed to direct sunlight in order to prevent rancidity of the oil in the feed. The formulated diets were kept in black polythene bags and stored in a dark, dry cupboard.

Experimental Design

Twenty (20) male rats (Wistar strain) were purchased from Anagonye's farm at Ifite Awka, Anambra State. They were placed in well ventilated cages at the Animal house of the Department of Applied Biochemistry Nnamdi Azikiwe University, Awka where feed and water were provided for them all through the period of the experiment. The rats were divided into four groups each containing 5 rats.

Animal grouping

After the acclimatization, Group A which is the control, received the formulated normal diet only. Group B were fed the formulated feed containing extra virgin olive oil . Group C received the formulated feed containing canola oil . Group D received the formulated feed containing sunflower oil all in the ratio of 4:1 (wt/wt) respectively. The experiment lasted for 21 days and the body weights of the rats were measured weekly using a digital weighing balance (Mettler balance).

Sacrificing animals and blood collection

After 21 days of feeding the animals, the animals were anesthetized after an overnight fast; they were dipped in a container containing cotton wool soaked with chloroform for few minutes until they were unconscious. Blood samples were collected by cardiac puncture and gently poured into both plain and anticoagulant bottles (for ESR) that were properly labeled for each group. The blood samples for SOD, Catalase, and MDA were centrifuged at 13,000rpm for 5 minutes to obtain sera.

Methods

Catalase activity Determination

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

Determination of Superoxide Dismutase (SOD) activity

Superoxide Dismutase activity was determined by the method of Sun and Zigma (1978).

Malondialdehyde (MDA) Content Determination.

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

Erythrocyte Sedimentation Rate (ESR)

The ESR was determined using the method described by Reza *et al.* (2015).

Statistical analysis

All data were represented as mean \pm SD. The mean values were statistically analysed using one way analysis of variance (ANOVA). P values less than 0.05 were considered significant.

Results

The results of the mean weight, catalase activity, SOD activity, MDA and ESR levels of the experimental animals are shown in fig 1,2,3,4, and 5 respectively.

Average 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 study.

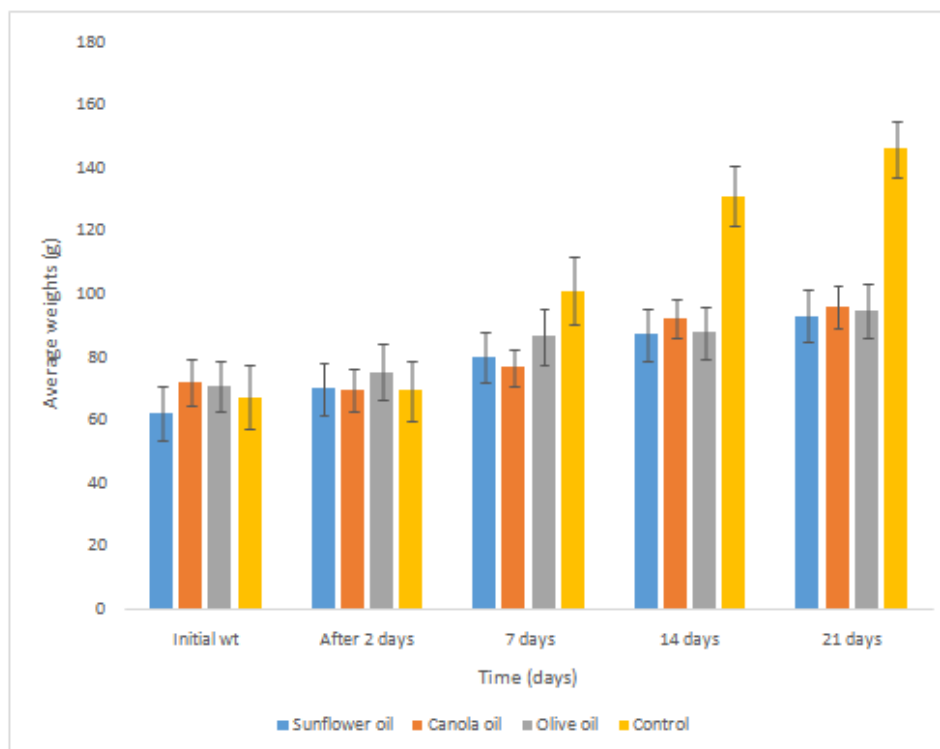


Fig 1: Average weights(g) of the experimental animals.

Average catalase activity:

The average catalase activity of the extra virgin olive oil group was comparable with that of the control group while those of the canola oil and sun flower oil groups were much higher (fig 2).

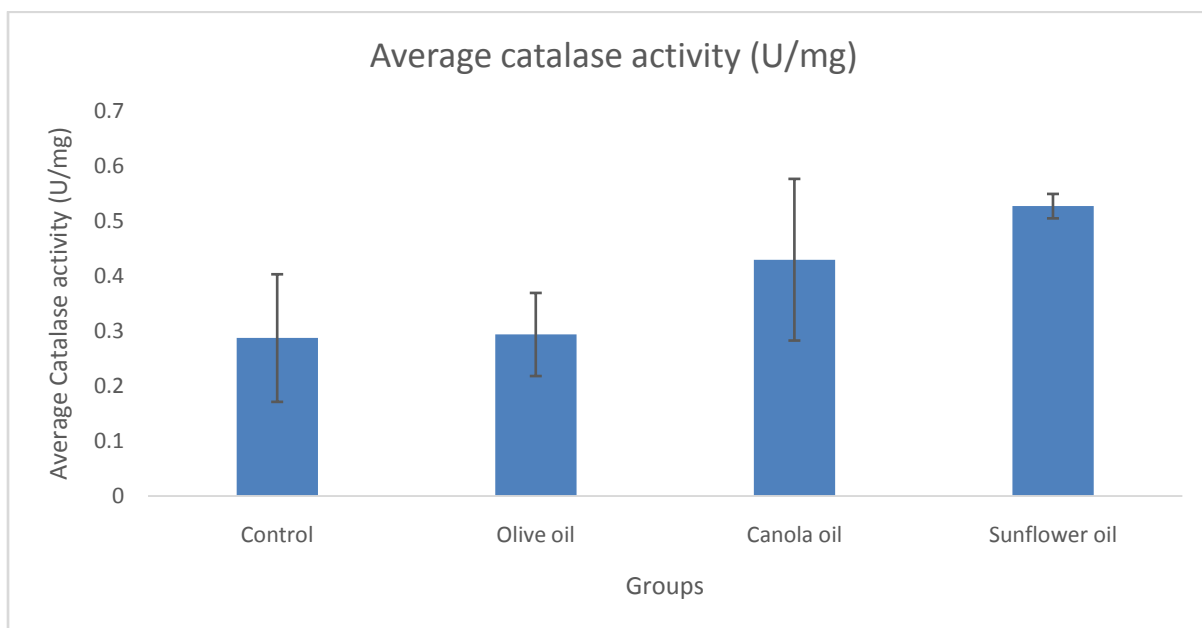


Fig 2: Average catalase activity (U/mg) of the experimental animals

Average SOD activity:

The average SOD activity of the extra virgin olive oil group was comparable with that of the control group while those of the canola oil and sun flower oil groups were much higher, with sunflower oil group recording the highest SOD activity (fig 3).

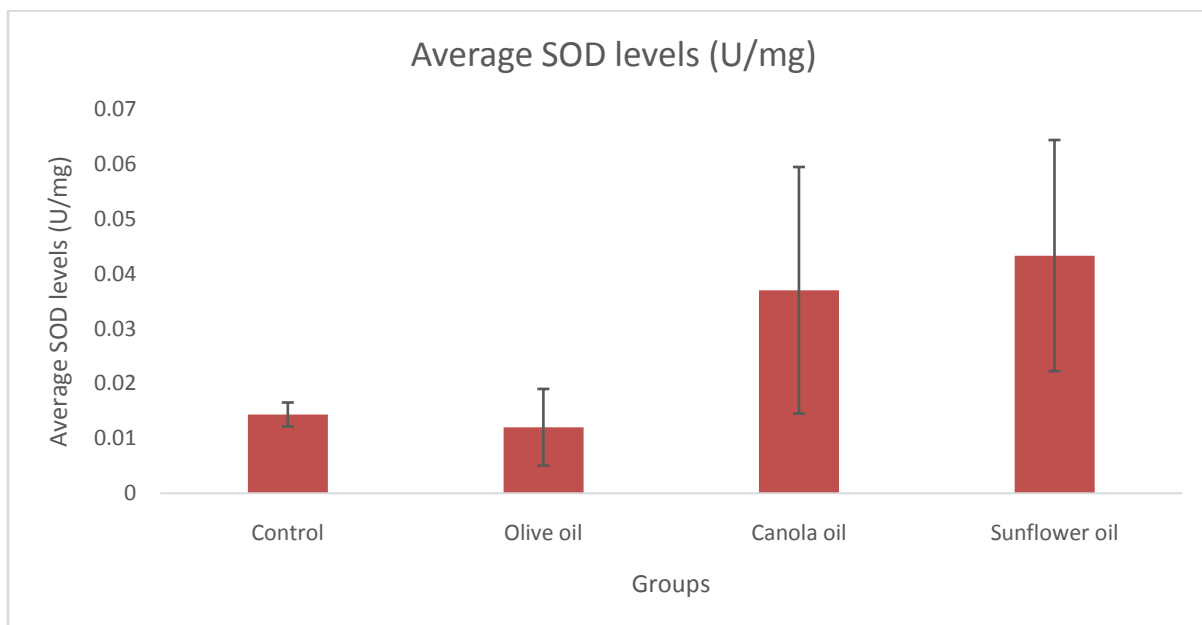


Fig 3: Average SOD activity (U/mg) of the experimental animals

Average MDA activity:

The average MDA activity of the extra virgin olive oil group was the lowest even lower than that of the control group while those of the canola oil and sun flower oil groups were much higher, with canola oil recording the highest MDA activity (fig 4).

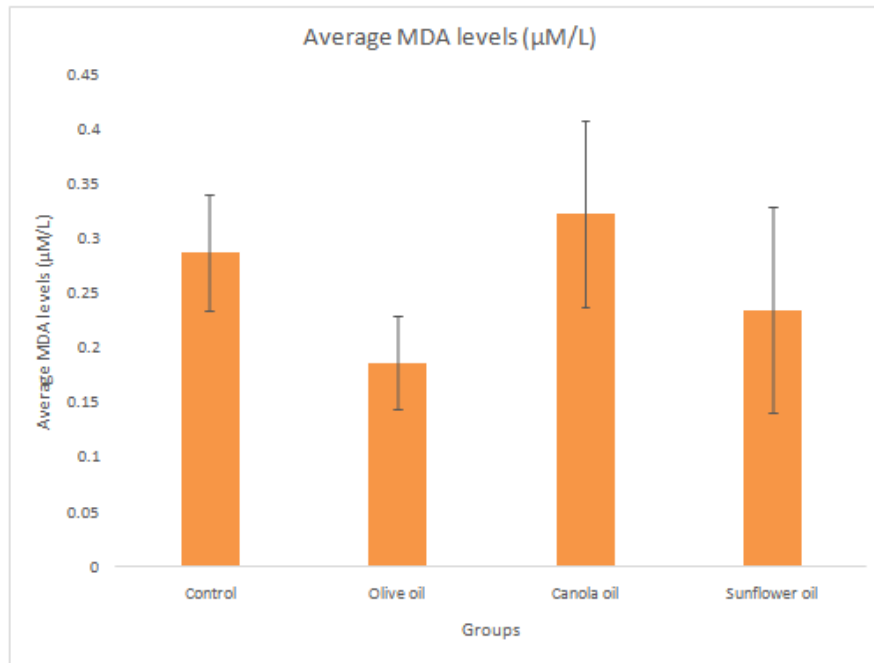


Fig 4: Average MDA levels (µM/l) of the experimental animals

Average ESR levels:

The average ESR activity of the extra virgin olive oil group was the lowest even lower than that of the control group while those of the canola oil and sun flower oil groups were much higher, with sunflower oil recording the highest MDA activity (fig 5).

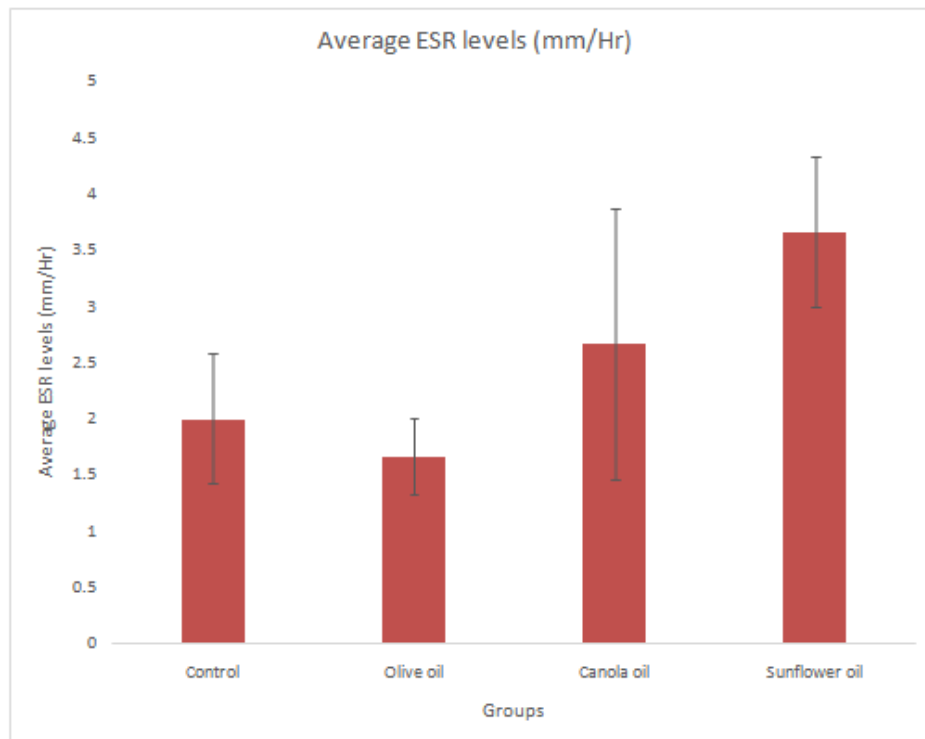


Fig 5: Average ESR levels (mm/hr) of the experimental animals

III. Discussion and conclusion

Discussion

This study investigated the effect of edible vegetable oils namely extra virgin olive oil, sunflower oil and canola oil on the serum inflammatory and lipid peroxidation markers of albino wistar rats fed with the oils. Results obtained showed that except for group B, all the groups showed significant increase ($P < 0.05$) between their mean initial and final weights. The catalase result for olive oil, canola oil and sunflower oil were 0.293 ± 0.075 IU, 0.429 ± 0.147 IU and 0.527 ± 0.022 IU respectively, but were not significantly different ($P > 0.05$) when compared to the catalase level in the control group. The superoxide dismutase activity (SOD) of rats fed with canola oil, sunflower oil as well as the control group were 0.037 ± 0.022 IU, 0.043 ± 0.021 IU and 0.014 ± 0.002 IU respectively which were higher than those fed with olive oil (0.012 ± 0.007 IU), but there was also no significant difference when the superoxide dismutase activity was compared in all the rat groups. There was no significant difference ($P > 0.05$) in both ESR and MDA levels in all the groups. Group C had the highest MDA levels of 0.324 ± 0.085 μ M which was higher than that of the control (group A) which had MDA levels of 0.288 ± 0.053 μ M; Group B had the lowest MDA levels of 0.188 ± 0.043 μ M; Group D had the highest ESR levels of 3.667 ± 0.667 mm/hr which was above that of the control which had ESR levels of 2.000 ± 0.577 mm/hr; Group B had the lowest ESR levels of 1.667 ± 0.333 mm/hr. Antioxidant enzymes control autoxidation by inhibiting the formation of free radicals via different mechanisms. A previous study by Gaschler and Stockwell, (2017) indicated that the presence of reactive oxygen species (ROS) in the system triggers the release of antioxidant enzymes which helps in scavenging the species that initiate peroxidation, breaking the autoxidative chain reaction, quenching O_2^- , and preventing the formation of peroxides.

Ohara et al, (2009) observed that the ingestion of canola oil increased the activity of SOD and Catalase when compared to the control.

Hamden et al, (2009) demonstrated that administration of EV olive oil increased the catalase activity in rat plasma, liver and kidney when compared to the control. Furthermore, Oliveras-Lopez et al, (2013), reported that administration of EV olive oil decreased the activity of SOD in humans and rat plasma especially at 25 days of treatment when compared to the control. It has been demonstrated that a Mediterranean diet enriched with extra virgin olive oil is beneficial for numerous cardio metabolic factors such as blood pressure, glycaemia, dyslipidemia (by decreasing triacylglyceride increasing HDL-cholesterol and lowering total and LDL-cholesterol) and additional risk factors such as oxidative stress (by reducing susceptibility of LDL to oxidation) and inflammation (by decreasing pro-inflammatory markers such as C-reactive protein and IL-6) (Zaineddinet al., 2012).

Lopez-Miranda et al., (2010) studied the inflammatory effects of vegetable oils on human health using experimental rats and found out that there was no significantly increased inflammatory markers after the feeding experiment, this goes in line with the results of this study as seen in the serum MDA and ESR levels of the experimental rats. High levels of MDA cause inflammation and can be related to a lot of cardiovascular diseases which can also be linked to increased weight gain or obesity.

IV. Conclusion

The edible vegetable oils investigated in this study belong to the monounsaturated (Extra Virgin Olive oil) and polyunsaturated (Sunflower and Canola oils). The results of the study showed that there were no significant differences in the inflammatory and lipid peroxidation parameters investigated among the different experimental groups. It is worthy of note that the inflammatory and lipid peroxidation parameters investigated were higher in the polyunsaturated oils (Sunflower and Canola oils) and lower in the monounsaturated oil (Extra Virgin Olive oil). Since we consume these oils for life the tendency is that an extended or prolonged study may find significant differences in the inflammatory and lipid peroxidation parameters between the two types of oils. There is need for consumers of these vegetable oils to balance the consumption of these oils.

References

- [1]. Attorri L., Di Biase A., Di Benedetto R., Rigato P., Di Virgilio A., Salvati S. (2010). Micronutrient-enriched rapeseed oils reduce cardiovascular disease risk factors in rats fed a high-fat diet. *Atherosclerosis*, 213: 422–428.
- [2]. Buege, J.A. and Aust, S.D. (1978). Microsomal Lipid Peroxidation. *Methods in Enzymology*. 52: 302-310
- [3]. Eichenfield LF, McCollum A, Msika P. (2009) The benefits of sunflower oleodistillate (SOD) in pediatric dermatology. *Pediatric Dermatology* 26(6): 669–675
- [4]. Emmanuel, O. A and Mudiakoeghene, O. (2008). "The Use of Antioxidants in Vegetable Oils – A Review," *African Journal of Biotechnology* 7(25): 4836-4842.
- [5]. Gaschler M.M, Stockwell B.R (2017). Lipid peroxidation in cell death. *Biochem. Biophys. Res. Commun.* 482: 419-425. 10.1016
- [6]. Gillingham, L.G., Harris-Jan, S. & Jones, P.J.H. (2011) Dietary monounsaturated fatty acids are protective against metabolic syndrome and cardiovascular disease risk factors. *Lipids* 46: 201–209
- [7]. Hamden, K, Allouche, N, Damak, M, and Elfeki, A. (2009). 'Hypoglycemic and antioxidant effect of phenolic extract and purified hydroxyl tyrosol from olive mill waste in vitro and in rat' *Chemo-Biological interaction*, 180 (3): 421-432.

- [8]. Hunter, J.E., Zhang, J.,and Kris-Etherton, P.M. (2010). Cardiovascular disease risk of dietary stearic acid compared with trans, other saturated, and unsaturated fatty acids: a systematic review. *Am J Clin Nutr.*;91:46–63.
- [9]. Lopez-Miranda J, Perez-Jimenez F, Ros E. (2010) Olive oil and health: summary of the II international conference on olive oil and health consensus report, Jaen and Cordoba (Spain) 2008. *Nutrition Metabolism Cardiovascular Diseases*20: 284–294
- [10]. Martin-Pelaez S, Covas MI, Fito M, Kusar A, Pravst I. (2013) Health effects of olive oil polyphenols: recent advances and possibilities for the use of health claims. *Molecular Nutrition and Food Research*57: 760–771
- [11]. Oguntibeju, O.O., Esterhuysen A. J and Truter. E. J (2010). "Possible Role of Red Palm Oil Supplementation in Reducing Oxidative Stress in HIV/AIDS and TB Patients: A Review," *Journal of Medicinal Plants Research*, 4(3): 188-196
- [12]. Ohara, N, Natio, Y, Nagata T, Saito Y, Kuwagata, M, Okuyama, H. (2009) Similar changes in clinical and pathological parameter in wister Kyoto rats after 13 week dietary intake of canola oil or fatty acid composition-based interesterified canola oil mimic. *Food and Chemical Toxicology*47: 157-162
- [13]. Oliveras-Lopez, M.J, Molina, J.J, Mir, M.V, Rey, E.F, Maetin, F, and De la serrana, H.L. (2013) 'Extra virgin olive oil consumption and antioxidant status in healthy institutionalized elderly human; *Achives of Gerontology and Geriatrics*.57: 234-242.
- [14]. Sinha, A.K. (1972). Colorimetric Assay of Catalase. *Anal. Biochem.*47(2): 389-394.
- [15]. Sun M, and Zigma S. (1978). An improved spectrophotometric assay of superoxide dismutase based on ephinephrineantioxidation. *Anal Biochem.*90:81-89.
- [16]. Reza H., Alireza M., Hassan M., Afshin A. Fares N. and Ali T. (2015). Erythrocyte Sedimentation Rate Measurement Using a Rapid Alternative to the Westergren Method. *Spring*3(2): 50-53
- [17]. Ukiya M, Akihisa T, Yasukawa K, Koike K, Takahashi A, Suzuki T. (2007) Triterpene Glycosides from the Flower Petals of Sunflower (*Helianthus annuus*) and Their Anti-inflammatory Activity. *Journal of Natural Products*70: 813–816
- [18]. Zaineddin AK, Buck K, Vrieling A, Heinz J, Flesch-Janys D, Linseisen J (2012) . The association between dietary lignans, phytoestrogen-rich foods, and fiber intake and postmenopausal breast cancer risk: a German case-control study. *Nutrition and Cancer*64(5): 652–65

Okafor, C.S, et. al. "Inflammatory and Lipid Peroxidationeffects of Canola Oil, Extra Virgin Olive Oil, and Sunflower Oil on Albino Rats Fed With the Oils." *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, 14(6), (2020): pp 33-39.