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Research Article

COMPARATIVE STUDIES ON *RICINUS COMMUNIS, ELAEIS GUINEENSIS* AND *HELIANTHUS ANNUUS* OIL CONSUMPTION ON ATHEROSCLEROSIS PATHOGENESIS USING ALBINO RATS

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Abstract

Consumption of saturated fat has been pointed out as one major contributor to the development of atherosclerosis. This study aims to investigate the comparative effects of castor oil (*Ricinus communis*), palm oil (*Elaeis guineensis*) and sunflower oil (*Helianthus annuus*) consumption on the atherosclerosis pathogenesis using albino rats. Castor oil, sunflower oil and palmoil were mixed with rat chow at varying percentages (2.5% - 10%) and fed to forty Wistar rats (male and female) weighing between 110g – 125g for 21 days. The rats were divided into groups labeled A through H, with five rats in each group. After 21 days, the body weight, liver function parameters, lipid profile, kidney function parameters, lipid peroxidation, and other biochemical analysis were determined using standard procedures. The results from the research showed significant weight gain in rats fed with sunflower oil and palm oil diets, while castor oil fed group led to a significant decrease in body weight. Liver function test revealed elevated alkaline phosphatase levels in groups B, C and E. Lipid profile results showed a significant increase($p < 0.05$) in total cholesterol level and non-significant increase in high-density lipoproteins and low-density lipoproteins in all groups. Kidney function tests indicated potential renal damage in group B. Malondialdehyde levels which indicated lipid peroxidation, showed a significant increase in group D. The different combinations of oils have varying effects on various physiological parameters, however, castor oil has a higher tendency to trigger the pathogenesis of atherosclerosis, especially at a 10% mix.

Keywords: Atherosclerosis, Triglycerides, Cholesterol, Low-density Lipoprotein.

INTRODUCTION

Cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vessels. An estimated 17.9 million people have been reported to have died from cardiovascular diseases in 2019, and this figure represents approximately 32% of all global deaths in that year (WHO, 2021). Isyaku *et al.* (2021) reported that three-quarters of these deaths occurred in low and middle-income countries such as in sub-Saharan Africa. Sub-Saharan Africa has witnessed a substantial increase of atherosclerotic cardiovascular diseases (ASCVD) over the last three decades (Neema *et al.,* 2022). Cardiovascular disorders are among the predominant causes of sudden, unexpected natural deaths in Nigeria (Okechukwu *et al.,* 2023) And worried by this development the Nigerian Heart Foundation advocated for a review of national guidelines for food production. Okechukwu *et al.* (2023) stated that CVDs now account for approximately 10% of deaths and 3.8% of disability-adjusted life years. Atherosclerosis is one of the leading causes of cardiovascular diseases worldwide. It is a multifactorial disease characterized by the accumulation of plaque within arterial walls, ultimately leading to narrowing of the blood vessels and restricting blood flow (Anastasia *et al.* 2020). It involves altered lipid metabolism, increased oxidative stress, impaired mitochondrial function, and chronic inflammation (Mathew *et al.* 2022). Atherosclerosis usually does not display signs and symptoms until it severely narrows or totally blocks an artery (Anastasia *et al.* 2022). Risk factors of atherosclerosis range from genetic conditions to environmental factors and modifiable lifestyle behaviours (Katharina *et al.,* 2020).

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Such factors include unhealthy diet, smoking, high blood pressure, diabetes, obesity. Dietary factors play a crucial role in the development and progression of atherosclerosis, with dietary oils rich in saturated fatty acid being implicated in its pathogenesis (Elisa *et al.,* 2021). Sunflower oil, Castor oil, and Palm oil have emerged as our prominent subjects of investigation due to their widespread consumption and varying fatty acid compositions. The Nigerian diet is characterized by high oil consumption, with palm oil being the most widely used. There has also been increasing popularity of alternative oils like castor oil and sunflower oil. Despite the widespread consumption of palm oil, castor oil, and sunflower oil in Nigeria, the country faces a high burden of cardiovascular disease. This lead the Nigerian Heart Foundation to advocate for a review of national guidelines for food production. There has been a growing body of literature on the impact of these oils on cardiovascular health of Nigerians. Our study intends to add to the growing body of literature that solves the challenge of developing evidence-based dietary recommendations for cardiovascular health promotion by comparatively looking at the effects of castor oil, sunflower oil and palm oil on cardiovascular health. Understanding the comparative effects of these oils on the progression of atherosclerosis is crucial as it reveals the intricate relationship between dietary components and cardiovascular health. The study aims to investigate the influence of dietary variables on the progression of atherosclerosis, with a specific emphasis on comparing the effects of various oils on its development. The comparative investigation will focus on *Ricinus communis* (castor oil), *Elaeis guineensis* (palm oil), and *Helianthus annuus* (sunflower oil) due to their unique compositions and potential impacts on atherogenesis. The findings of this study may provide valuable insights for dietary recommendations aimed at maintaining cardiovascular health.

MATERIALS AND METHODS

Sample collection

Palm oil and Sunflower oil (Saffola sunflower oil) were sourced from Eke Awka market in Awka, Anambra state. The castor oil (Finest cold drawn) was sourced from Abel and Jane pharmacy, Oko, Orumba North L.G.A. Anambra state.

Feed formulation

Different percentages of the various oils were mixed with the rat chow and assigned to their respective groups.

Animal studies

Forty (40) adult Wistarrats (male) weighing 110-125g were purchased from Chris farms, Mgbakwu town, Awka North, L.G.A, Anambra State. The rats were kept in standard cages with sawdust as bedding and standard housing condition. The rats were acclimatized for one week before the feeding study.

Ethical approval

All experiments were approved by the Nnamdi Azikiwe University Animal Research Ethics Committee (NAU-AREC) in line with the principles of Animal Care and Use in Research, Education and Testing. The ethical approval number as issued by the NAU-AREC is NAU/AREC/2024/0019.

Experimental design

The rats were separated into eight groups (A - H) of fiverats per group. The castor oil, sunflower oil and palm oil were used as fatty acid source in the formulated feeds and fed the test animals. The control experimental animals were fed with rat chow without oil. The animals were allowed access to feed and water for twenty-one (21) days.

Group A: Normal Control (100g rat chow)
Group B: Rat Chow (90g) + 10% castor oil
Group C: Rat Chow(90g) + 10% Palm oil
Group D: Rat Chow (90g) + 10% Sunflower oil
Group E: Rat Chow (90g) + 5% castor oil + 2.5% palm oil + 2.5% sunflower oil
Group F: Rat Chow (90g) + 5% palm oil + 2.5% castor oil + 2.5% sunflower oil
Group G: Rat Chow (90g) + 5% Sunflower oil + 2.5% castor oil + 2.5% palm oil
Group H: Rat Chow (90g) + 3.33% castor oil + 3.33% palm oil + 3.33% Sunflower oil

Determination of bodyweight

The animals were weighed using electric weighing scale (G & G JJ300, China) before the feeding study and at the end of the each week during the feeding study.

Sacrifice and sample collection

After twenty one (21) days, the rats were anaesthetized with chloroform and blood was drawn through cardiac puncture. Blood samples were dispersed into universal bottles for analysis.

Biochemical analysis

Assay of Haematological Parameters

Hematological parameters such as hemoglobin concentration (HGB), packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC) and platelets counts were estimated using Mindray Hematology Autoanalyser (BC-2800, India).

Liver Function Tests

The blood was centrifuged at 4000rpm with a centrifuge (PEC Medical, USA) for 15mins and the serum was used for the assay. The liver function test was conducted using the method of Limdi and Hyde (2003).

Aspartate Amino transferase (AST)

The Aspartate Amino transferase was determined by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine at 546nm. An Aliquot of the serum (0.1ml) was mixed with 0.5ml of Randox AST R1 buffer containing 100mmol phosphate buffer, 100mmol L-aspartate and 2mmol α-oxoglutarate. This was allowed to stand for 30mins at room temperature followed by the addition of 0.5ml of 2mmol 2, 4-dinitrophenylhydrazine. After 20mins, 5ml of 0.4M NaOH was added and the absorbance was taking at 546nm after 5min. the concentration of AST in the serum was calculated from the standard values given by Randox.

Alanine Amino transferase (ALT)

The Alanine Amino transferase was determined by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine at 546nm. An Aliquot of the serum (0.1ml) was mixed with 0.5ml of Randox ALT R1 buffer containing 100mmol phosphate buffer, 200mmol L-alanine and 2mmol α-oxoglutarate. This was allowed to stand for 30mins at room temperature followed by the addition of 0.5ml of 2mmol 2, 4-dinitrophenylhydrazine. After 20mins, 5ml of 0.4M NaOH was added and the absorbance was taking at 546nm after 5min. the concentration of ALT in the serum was calculated from the standard values given by Randox.

Alkaline Phosphatase Assay

In the assay, 20µL of the serum was mixed with 1ml of 10mmol/L pnitrophenylphosphate in 1mol/l Diethanolamine buffer. The initial absorbance was read immediately with Axiom 752 UV-VIS at 405nm, and then the absorbance was taken again after 1min, 2min and 3mins.

The ALP activity was calculated as follows:

$ALP\ (U/L) = 2760\Delta A405nm/min$

Where $\Delta A405$ = change in absorbance at 405nm.

Assay of the Lipid Profile

The lipid profile (Total Cholesterol, Triglycerides, High-Density Lipoprotein-cholesterol, Low-Density Lipoprotein-cholesterol and Very Low-Density Lipoprotein-cholesterol) were determined using Randox test kits (Trinder, 1969 and Tietze *et al.,* 1990). Low-density Lipoprotein-cholesterol (LDL-c) was calculated using a standard formula (Friedwald *et*

al. 1972). The procedure used was according to the manufacturer's instructions provided in the manual.

Kidney Function Test

Serum Creatinine: Creatinine was determined based on its reaction with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration. An aliquot of the serum (50µL) was mixed with 0.5ml of randox reagent containing 35mmol/l picric acid and 0.32mol/l NaOH. This was read with autoanalyzer and the concentration of creatinine displayed on the machine was recorded.

Serum Urea: Urea in the serum was hydrolyzed to ammonia in the presence of urease. The Ammonia liberated was measured photometrically by Berthelot's reaction. An aliquot of the serum (5µL) was mixed with 50µL of Randox reagent containing 116mmol/L sodium nitroprusside and 6mmol/L urease. It was allowed to stay for 10mins at 370C after which 1.25ml of 120mmol/l phenol and 27mmol/l sodium hypochlorite was added and all owed to stay for 15mins at 37oC. The Malondialdehyde concentration of urea was then recorded with auto analyzer.

Determination of lactate dehydrogenase Activity

A 1.0 ml of buffered substrate and 0.1 ml of sample were placed into each of the two tubes. 0.2 ml of water was added to the blank. Then to the test, 0.2 ml of NAD, mixed and incubated at 37oC for 15min. After15 min, 1.0 ml of dinitrophenyl hydrazine was added to each (test and control). Left for further 15m min. Then 10 ml of 0.4N sodium hydroxide was added and the color developed was read immediately at 440nm. A standard curve with sodium pyruvate solution with concentration range 0.02 – 0.10 µmol was taken. LDH activity in the serum is expressed as µmoles of pyruvate liberated/L and in tissue homogenate as nanomoles of pyruvate liberated/min/mg protein.

Lipid Peroxidation Determination

Lipid peroxidation was determined by the thiobarbituric acid-reacting substances (TBARS) method by Buege and Aust (1978). The serum 0.4ml was collected into the test tube, 1.6ml of 0.25N HCL was added together with 0.5ml of 15% trichloroacetic acid and 0.5ml of 0.375% thiobarbituric acid and then mixed thoroughly. The reaction mixture was then placed in 100 boiling water for 15 minutes, allowed to cool and centrifuged at 3000 revolution per minute for 10 minutes. The supernatant was collected and the optical density was recorded at 532nm against reagent blank containing distilled water. The lipid peroxide activity was calculated using the formula

Lipid peroxide activity = Optical density/Time × Extinction coefficient/Amount of sample

Where the extinction coefficient value is 1.56 × 10-5M-1 /cm-1, Optical density =532nm, Time = 15mins. Amount of sample = 15mg. The unit is expressed as µmol/MDA/mg of serum.

Statistical Analysis

Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences software for windows version 23 (SPSS Inc., Chicago, Illinois, USA). All the data collected were expressed as Mean ± SEM. Statistical analysis of the results obtained were performed by using ANOVA Tests to determine if a significant difference exists between the mean of the test and control groups. The limit of significance was set at $p<0.05$.

RESULTS

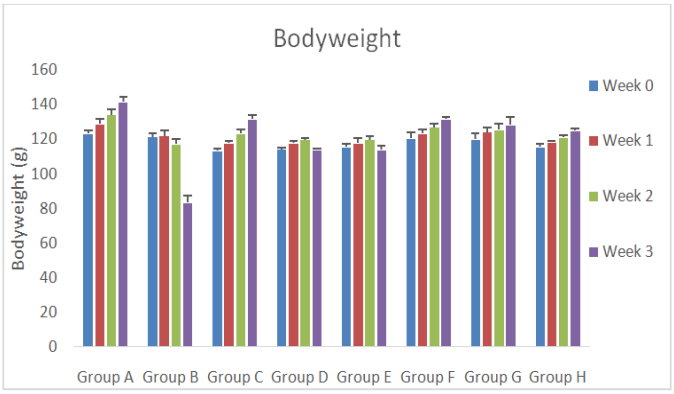

Fig. 1. Effects of feeding with different percentages of castor oil, palm oil and sun flower oil on bodyweight of wistar rats

The result indicated that body weight increased in all groups except for group B. Significant weight gain was observed in groups A, C and F at week 3 when compared to week 0. Group B showed a significant decrease in bodyweight when compared to its bodyweight at week 0. Groups with the combination of the three oils at varying percentages recorded gradual weight increase.

In the haematology result, haemaoglobin (HGB), packed cell volume (PCV), and red blood cell count (RBC) show no significant difference ($p < 0.05$) in the various groups fed with different percentages of the various oils when compared to the control group. In the platelet count, the result shows a significant increase ($p < 0.05$) in group B (809.00 ± 75.00), C (658.67 ± 52.30), F (652.00 ±9.07) and G (655.33 ±50.32) when compared to the control group (489.67 ±118.92).

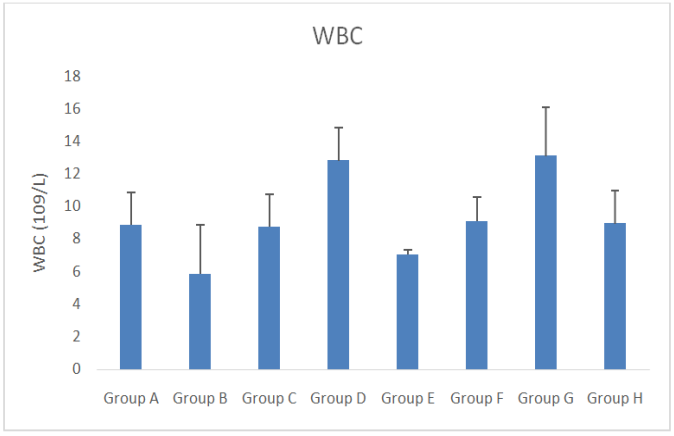

Fig. 2. Effect of feeding with different percentages of castor oil, palm oil and sunflower oil on White blood cell count of wistar rats

The result of the white blood cell (WBC) count showed increases which were not statistically significant ($p < 0.05$) when compared to the control group.

Table 1. Results of the effect of feeding with different percentages of castor oil, palm oil and sun flower oil on haematological parameters of wistar rats expressed as mean ± SEM

Groups	HGB (g/dl)	PCV (%)	RBC (10^{12}/L)	PLT (10^{9}/L)
Group A: Normal Control	13.30 ± 0.92	36.47 ±10.56	6.43 ± 0.37	489.67 ± 118.92
Group B: Rat Chow + 10% CO	14.10 ± 0.40	43.00 ± 1.00	6.81 ± 0.21	809.00 ± 75.00[a]
Group C: Rat Chow + 10% PO	14.17 ± 0.49	42.90 ± 1.25	6.76 ± 0.09	658.67 ± 52.30[a]
Group D: Rat Chow + 10% SFO	12.43 ± 0.13	39.23 ± 0.44	6.08 ± 0.21	556.33 ± 104.64
Group E: Rat Chow + 5% CO + 2.5% PO + 2.5% SFO	12.93 ± 0.15	40.40 ± 0.53	6.26 ± 0.08	551.67 ± 13.54
Group F: Rat Chow + 5% PO + 2.5% CO + 2.5% SFO	13.93 ± 0.90	42.90 ± 2.12	6.50 ± 0.14	652.00 ± 9.07[a]
Group G: Rat Chow + 5% SFO + 2.5% CO + 2.5% PO	12.63 ± 1.07	39.33 ±3.28	6.72 ± 0.17	655.33 ± 50.32[a]
Group H: Rat Chow + 3.33% CO + 3.33% PO + 3.33% SFO	10.30 ± 0.60	31.87 ±1.99	5.40 ± 0.17	577.33 ± 37. 29

[a]Significant increase with respect to normal control. CO: Castor Oil, PO: Palm Oil, SFO: Sun Flower Oil.

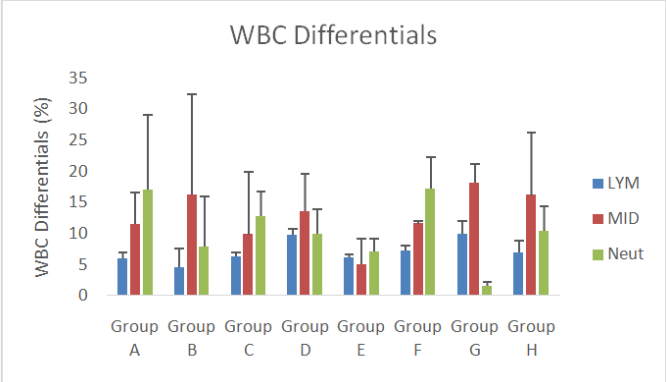

Fig 2. Effect of feeding with different percentages of castor oil, palm oil and sunflower oil on White blood cell differentials of wistar rats

The result of white blood cell differential shows no significant difference between the various treatment groups and the control group.

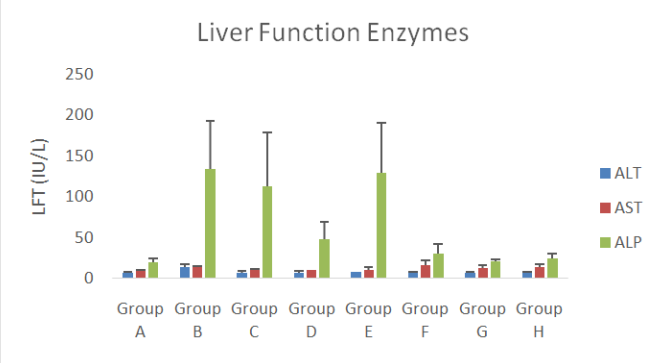

Fig. 4. Effects of feeding with different percentages of castor oil, palm oil and sun flower oil on liver function parameters

The result of liver function test showed no significant difference in Aspartate aminotransferase (AST) of the test groups when compared to the control group. There is a significant increase ($p < 0.05$) in Alanine transaminase (ALT) of group B when compared to the control group. The result showed significant increase in Alkaline phosphatase (ALP) of groups B, C, and E. Results of the lipid profile showed a significant decrease ($p < 0.05$) in group B when compared to the control. The HDL levels ofthe other groups showed a decrease but not statistically significant when compared to the control. In the total cholesterol (TCHOL) result, group C showed a significant increase when compared to the control group. There were increases in the other groups but not statistically significant ($p < 0.05$) when compared to control group. Groups D and F, showed significant increase ($p < 0.05$) in their Triglyceride (TRIG) levels.

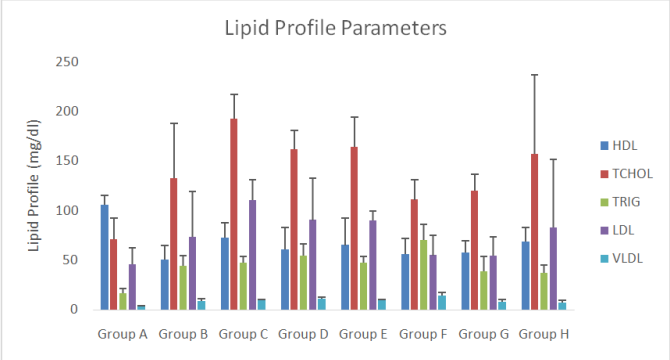

Fig. 5. Effects of feeding with different percentages of castor oil, palm oil and sunflower oil on lipid profile parameters

In the other groups, there were increases in the Triglyceride levels but not statistically significant. Low density lipoproteins (LDL) levels showed increases in all groups but not statistically significant ($p < 0.05$) when compared to the control group. The result of very low density lipoproteins (VLDL) showed significant increases in groups D and F. The other groups showed increases which were not statistically significant.

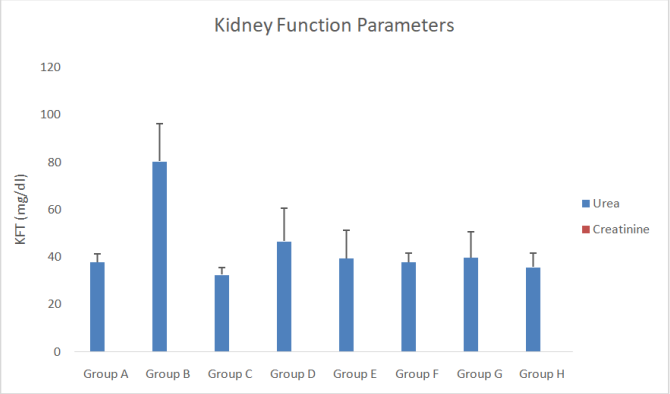

Fig 6. Effects of feeding with different percentages of castor oil, palm oil and sunflower oil on kidney function test expressed as mean ± SEM

Investigation of the kidney function showed that there is a statistically significant increase ($p< 0.05$) in mean values of Urea and creatinine in group B when compared to the control group. The mean value of urea and creatinine levels in other treatment groups showed no significant difference when compared to the control group.

In our result of lactate dehydrogenase activity, Group B and G showed a significant increase when compared to the control. Groups D, E, F, and H showed ria se in lactate dehydrogenase activity but were not statistically significant.

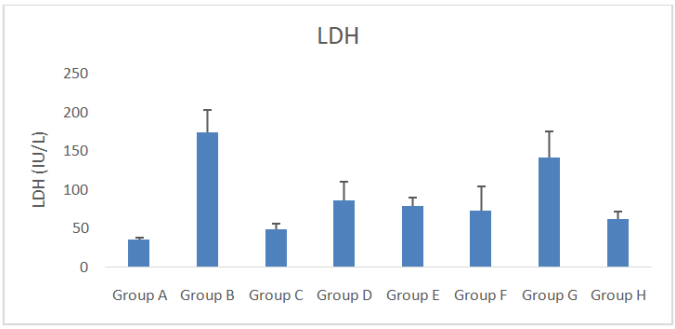

Fig. 7. Effects of chow feeding with different percentages of castor oil, palm oil and sun flower oil on lactate dehydrogenase enzyme expressed as mean ± SEM

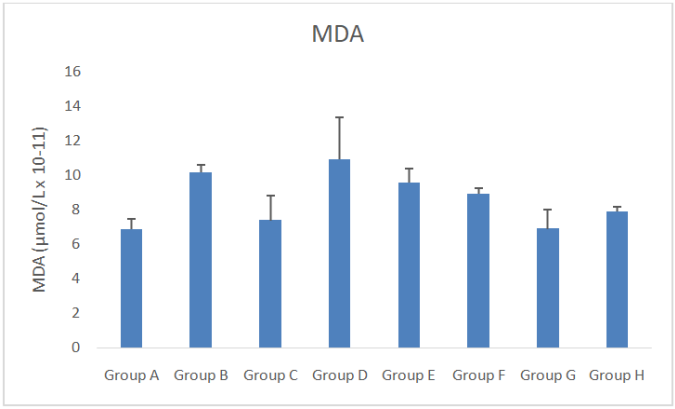

Fig. 8. Effects of chow feeding with different percentages of castor oil, palm oil and sun flower oil on malondialdehyde expressed as mean ± SEM

The result of the production of malondialdehyde showed that there was no significant difference observed between test groups and control except for group D which showed a significant increase ($p<0.05$).

DISCUSSION

The results of body weight indicated that body weight increased in all groups except for group B. Significant weight gain was observed in groups A, C, and F at week 3 when compared to week zero (0).This is in agreement with the findings of Umezulike *et al.,* (2021). She reported a significant increase in body weight of various oil diet-fed groups. However, it is at variant with the findings of Tarfa *et al.,* (2022) who reported no significant weight gain in body weight of rats fed with palm oil or olive oil diets. Conversely, group B exhibited a significant decrease ($p< 0.05$) in body weight at week 3 when compared to its initial body weight at week zero (0).Non-significant decrease was observed in groups D and E when compared to their body weight at week zero (0). This decrease in body weight in group B could be attributed to the laxative properties of castor oil. Castor oil is rich in ricinoleic acid and has been widely used in bioassays involving anti diarrhea activity in laboratory animals due to its intestinal activity (Hamidu *et al*., 2020). In our haematology study, feeding on the various oils-supplemented diet did not result in significant changes in haemoglobin concentrations, packed cell volume, white blood cell, white blood cell differentials and red blood cell count. This however is contrary to the result of Imoh *et al.,* (2023) who reported a significant increase ($p > 0.05$) in haemoglobin concentration, Red blood cell count, platelet and pack cell volume in the palm oil-supplemented diet-fed group compared to the control group of his study on the comparative effects of fresh palm oil on haematological parameters of albino rats. Manal *et al.,* (2020) reported a significant decrease in haemoglobin, packed cell volume and red blood cell count in Wistar rats fed with sunflower-supplemented diets. The findings of Imoh *et al.,* (2023), agreed with our result on platelet count. He reported a significant increase in platelet count of the palm oil-fed group of his study when compared to the control. Platelet count in our study, showed a significant increase ($p < 0.05$) in Groups D, E, and H. Platelets play an important role in atherosclerosis development. An increase in platelet count favours the progression of atherosclerosis. They adhere to the damaged areas and release substances that promote blood clotting and inflammation and may lead to the formation of atherosclerotic plaques.

The result of liver function test revealed elevated alkaline phosphatase levels in groups B, C and E. The changes in the serum enzyme activities are important indicators in the early diagnosis of diseases or tissue damage due to toxic substances. The risk of atherosclerosis tends to increase with increasing ALP levels (*Lai et al.*, 2023).The notable elevation in alkaline phosphatase (ALP) and aspartate aminotransferase (AST) levels in our study may be attributed to the proportion of the various oils integrated into the diet which may have caused stress or damage to the liver. Castor oil ingestion is typically advised in moderation; as excessive consumption may adversely affect the health of the consumer. Tarfa *et al*., (2022) reported a significant decrease in ALT and AST levels in the palm oil-fed group of their study. Tesaka *et al.,* (2020), reported a significant increase in AST and ALT levels in their study of the effects of palm oil fried street kokor on liver biomarkers. These are contrary to our findings that showed no significant difference in ALT and AST of the palm oil-fed group.

According to Linton *et al.,* (2023), epidemiologic studies have shown an inverse relationship between HDL cholesterol (HDL-C) levels and atherosclerotic cardiovascular disease. High levels of HDL cholesterol can lower your risk for heart disease and stroke. The result of the total cholesterol showed that only animals in group C displayed an elevated cholesterol level that is statistically significant ($p<0.05$). This agrees with the reports of Essien *et al.,* (2021) and Tarfa *et al.,* (2022) where the total cholesterol of the treatment groups was higher than that of the control groups. According to Lee (2022), high cholesterol; among other conditions, damages the inner lining of blood vessels called the endothelium. The damage of the endothelium over time contributes to the build-up of cholesterol plaques and inflammatory cells in the blood vessels throughout the body, this condition is known as atherosclerosis (Lee, 2022). There were increases which were not statistically significant in the LDL result. Group C has the highest LDL level. LDL plays a central role in the development of atherosclerosis, carrying cholesterol from the blood and penetrating the endothelial lining of the arterial wall. Pratyush and Nishikant (2023) stated that vLDL causes plaque build-up in arteries if in excess. Therefore, the animals in groups D and F face the risk of atherosclerosis development. The result of our study revealed that there's no significant difference between the various groups and the control except for group B which showed a significant increase in Creatinine level. This is in disagreement with Tesaka *et al.,* (2020), who reported a significant increase in creatinine and urea levels in kidney biomarkers of rats fed with palm oil fried kokor. Our study

suggests that there is no manifestation of kidney damage in the palm oil diet-fed group. The significant increase observed in group B may be an indication of kidney damage as a result of castor oil consumption. Xin *et al.,* (2023) showed that serum creatinine was associated with several traditional cardiovascular risk factors in their studies on hypertensive patients. In our study, group B showed a significant increase in creatinine levels when compared to the control group. This increase can be due to the percentage of castor oil in the diet. Several literatures advise a minimal consumption of castor oil as excessive consumption could be detrimental.

Wenfang *et al.,* (2022) pointed out that increased Lactate dehydrogenase (LDH) levels could be a predictor of cardiovascular risk and arterial stiffness in health examined population. Lactate dehydrogenase is an important enzyme in anaerobic metabolism. From the submission of Zeng *et al.,* (2022), that increase in the level of LDH may be a robust predictor of cardiac risk. We see from our study that groups B and G showed an elevated LDH level and hence may likely aid the development of atherosclerosis. This increase may likely be an indication of tissue damage due the oil consumption. The oxidative modification of low-density lipoprotein (LDL) is considered a crucial step in the pathogenesis of atherosclerosis (Jan *et al.,* 2020). This process is driven by free radicals and results in the formation of lipid peroxides, which modify the LDL apoprotein through the action of aldehyde products derived from lipid peroxidation. Result from our study showed a significant increase in malondialdehyde concentrations of group D. This is in agreement with Gholamalian *et al.* (2022), who reported a significant increase ($P < 0.05$) in malondialdehyde concentrations in their study when they fed sunflower oil supplemented diet to the laying hens. The possible inference to be drawn is LDL particles rich in polyunsaturated fats are more readily oxidized, and this could have contributed to the increased rate of lipid peroxidation. The reason for the non-significant effect observed from the palm oil formulated feed fed group can be attributed to the vitamin E present in palm oil which prevented lipid peroxidation. Some studies suggest that castor oil may have anti-inflammatory and antioxidant properties.

Conclusion

The different combinations of oils have varying effects on various physiological parameters, however, castor oil has a higher tendency to trigger the pathogenesis of atherosclerosis, especially at a 10% mix. This is as a result of the Inflammatory effect of the ricinoleic acid content of castor oil. Palm oil follows due to its high content of total cholesterol and lastly sunflower oil.

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