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Formulation of Multi-Source Edible Oils from Palm oil and African Walnut oil and Study of Their Effect on Hematological, Inflammatory and Oxidative Stress Markers in High Fat Diet Obese-Induced Wistar Rats

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ABSTRACT

The objective of this study was to evaluate the effects of palm oil, African walnut oil and their blends on hematological, inflammatory, and some oxidative stress markers in high fat diet (HFD) obese-induced Wistar rats. Obesity was induced for 60 days and treated for 28 days using edible oils [palm oil, African walnut oil, palm oil: African walnut oil (50:50) and palm oil : African walnut oil (60:40)] and orlistat (10 mg/Kg). Thereafter the animals were sacrificed, blood was collected for hematological studies and the preparation of the serum, while the organs harvested were used to prepare organ homogenates. Serum and organ homogenates were used for the evaluation of inflammation and oxidative stress markers. Results showed that the oils utilized were confirmed to be of high quality through their good stability indices (peroxide value: 2.52-3.87meq O_2/Kg; *p*-anisidine value: 8.24-12.33, TOTOX value: 13.37-19.46,). Looking at the haematological study, animals that received the HFD presented the lowest ($p<0.05$) hematocrit and Platelet. PO:WO (50:50 and 60:40) significantly ($p<0.05$) decreased the granulocytes concentration in the blood of rats. PO:WO (50:50) significantly ($p<0.05$) increased the lymphocyte concentration while 100% PO increased the mid-size white blood cells level in the animals. Serum levels of inflammation markers were higher ($p<0.05$) in the negative control group (354.44-385.82 pg/mL) compared to the other groups (147.22-271.55 pg/mL). The analysis of oxidative stress parameters revealed that the administered oils and orlistat generally exhibited good protections compared to the normal and negative control groups, which might be due to the presence of omega-3 fatty acids and bioactives such as β-carotene and vitamin E which have good antioxidant and anti-inflammatory properties. It can be concluded that these oils have a role in protecting against obesity through their effects on oxidative stress, hematology, and inflammatory cytokines.

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Introduction

Over the last few years, the consumption of fast foods, high calorie foods, and processed foods has become a day-to-day activity resulting in medical conditions that show very clearly the implication of diet on health. Oils, being an essential and staple component of diet play a great role in an individual's overall health, as the type of fat consumed, method of preparation and frequency can ameliorate or deteriorate one's health. Obesity, a metabolic disorder characterized by fat accumulation in adipose tissues, has become increasingly prevalent and it is due to many factors amongst which to the consumption of high calorie foods, sedentary lifestyle and lack of exercise (Kelly & Uadia, 2020).

In recent years, the values representing statistics of obesity on a regional, national and international scale have been increasing. Statistics show that in 2022, one in eight people in the world were living with obesity. Obesity in adults has doubled since 1990 and has quadrupled in adolescent. Still in 2022, 2.5 billion adults (>18 year old) were overweight, from which 890 million were obese. 37 million children under age of 5 were reported to be overweight. Over 390 million children and adolescent aged 5-19 years were reported overweight in 2022, with 160 million of them living with obesity (WHO, 2024). The prevalence to obesity and overweight has risen worldwide in the pass decades also. According to the World Obesity

Atlas (2023), the estimated statistic of obese and overweight people in 2020 among the global population aged >5 was 38% which is expected to move to 51% by 2035 (Jeon et al., 2024).

According to a multicentric study, the prevalence of obesity in Africa ranges from 4.5 to 32.5% (Oladeji et al., 2021). Cameroon is not spared from this. The most recent data shows that the prevalence of obesity in Cameroon is approximately 9.4% among adults (WHO, 2015).

Obesity has been proven to be a major risk factor for developing several chronic diseases such as type 2 diabetes mellitus, cardiovascular diseases, cancer, kidney failure and so on because fat accumulation leads to complications affecting blood flow, insulin sensitivity, lipid metabolism, inflammatory responses and more (Shalom et al., 2020). According to WHO statistics from 2019, unhealthy fat intake and accumulation are the highest risk factors for developing heart diseases and stroke. In spite of the preventive measures put in place, the prevalence of obesity continues to increase and if this trend persists it is estimated that by 2025, 18% of men and 21% of women worldwide will be obese (Kinlen et al., 2018). Unhealthy dietary habits, particularly the consumption of high-fat diets is becoming rampant and plays a significant role in the development and progression of obesity.

The detrimental effects of excessive fat intake are well-documented (Schmidt et al., 2015). Consequently, there is a growing interest in exploring alternative dietary approaches that can mitigate the adverse effects of high-fat diets and their complications especially in developing countries like Cameroon, where consumption of pork, refined sugars and processed foods is on a rise. The Food Safety and Standards Authority of India (FSSAI) as of June 2021 recommended the consumption of balanced and diverse diets for maintaining optimal health (FSSAI, 2021). In line with these guidelines, the formulation of multi-source edible oils using a combination of two or more oils has gained attention due to its potential to provide a balanced fatty acid profile and a range of health benefits as oil blends are richer in nutrients especially in essential fatty acids (EFA), vitamins and are more stable than oils rich in polyunsaturated fatty acids alone (FSSAI, 2021; Dhyani et al., 2022).

Many oils are available in Cameroon and used individually with the most common being palm oil and its refined fraction-palm olein, which are consumed in all regions of Cameroon. Since the population is slowly becoming aware of the importance of consuming omega 3 and 6 rich oils, the oils available like soya bean oil and cotton seed oil are quite expensive and not accessible to everyone. Additionally, these oils are poor in omega 3 and omega 6 fatty acids. On the other hand, good plant sources of omega-6 and omega-3 fatty acids are available, that is the case of African walnut oil which contains about 14.41% of omega 6 fatty acids and 70.39% of omega 3 fatty acids. It will be important to evaluate the effect of the mixture of this oil with palm oil at different proportions according to FSSAI (2021) to see its effect on obesity.

Due to the rapid increase in the obese population, many drugs have been developed for their treatment and management purposes (for example Orlistat) but just like other synthetic drugs, these drugs are not always 100% effective, they are expensive, and may have side health effects depending on the individual (Yael et al., 2021). Orlistat was reported as very effective and well tolerated anti-obesity drugs which can be employed as adjunct to therapeutic lifestyle changes to achieve and maintain optimal weight (Jain et al., 2011). It acts by binding to serine residue found at the active site of gastric and pancreatic lipases. It acts by partially inhibiting the breakdown of triglycerides, thus reducing the absorption of free fatty acids and monoglycerides (Guerciolini, 1997). Diets rich in polyunsaturated fatty acids have proven to have very promising effects in combating non communicable diseases like obesity, and obesity related conditions (Duo, 2015). Since Cameroon has shown limited progress in using food ingredients and diets to address non-communicable disease (NCD), it is necessary to formulate and adopt diet changes using food staples that are readily available in Cameroon for obesity management (African Population and Health Research Center, 2018).

Palm oil is a widely consumed oil in all regions of Cameroon. It contains a balanced proportion of saturated fatty acids; particularly palmitic acid (44%), stearic acid (4.5%) and unsaturated fatty acids; oleic acid (39.2%), linoleic acid (10%), α-linolenic acid (0.4%). It also contains a high proportion of antioxidants, Vitamin E and Beta carotene (Annamaria et al., 2015). However, this oil was reported to inflame white adipose tissue and triggers metabolic disturbances in mice on a high-fat diet (Martins et al., 2024). Widjaja et al. (2018) reported that supplementation of coconut oil and palm oil in rats can increases body mass index, abdominal circumference, and fat mass. The effects recorded by these authors were related to the high content in saturated fatty acids of palm oil. On the other hand, omega-3 rich oils were reported to have improved body composition and counteract obesity-related metabolic changes (Albracht-Schulte et al., 2018). An example of omega-3 rich oil available in Cameroon and that can be exploited towards this direction is African walnut oil.

African walnut (*Tetracarpidium conophorum*) is a traditional oil source in Africa which has not been much exploited. It is highly available in Cameroon between July and September of each year and is often boiled, cracked and eaten like other traditionally boiled nuts. It is not exploited for its oil in Cameroon though it has been reported to contain 9.48% Oleic acid, 14.41% linoleic acid and 70.39% alpha-linoleic acid (Tchiegang et al., 2007). Similar composition was reported by Ghomdim et al. (2024) who showed that *Tetracarpidium conophorum* oil is composed of 14.35% oleic acid, 10.56% linoleic acid and 69.90% alpha-linolenic acid. African Walnuts was reported to attenuate ectopic fat accumulation and associated peroxidation and oxidative stress in monosodium glutamate-obese Wistar rats (Uti et al., 2020a). These same authors showed that African Walnuts modulate hepatic lipid accumulation in obesity via reciprocal actions on HMG-CoA reductase and paraoxonase (Uti et al., 2020b). It was also reported to have good hypolipidemic properties (Oriakhi and Uadia, 2020). Due to the fact that palm oil is the most consumed and available oil in Cameroon, blending it with omega-3 fatty acids-rich oil such as African walnut oil can help improve the health of obese patients. Multi-source edible oils or oil

blends from Palm and African Walnut oils can be a promising solution to reduce the prevalence of obesity in Cameroon if they are known and adopted by the population. Both oils have shown promising effects in various studies for their potential to modulate physiological processes and provide protective effects against certain diseases (Kaur et al., 2014). It can be hypothesized that multi-source edible oils from palm and walnut oils can significantly reduce the prevalence of obesity and some of its complications in high fat diet induced-obese Wistar rats. The objective of this study was to evaluate the effects of multi-source edible oils from palm and walnut oils in the management of obesity and some of its complications in high fat diet induced-obese Wistar rats.

Materials and Methods

Materials

Fresh African walnuts were purchased from a farmer in Muyuka, Fako Division, South-West Region of Cameroon in October 2023. 5 litres of pressed palm oil was purchased from Fontem, Lebialem Division, South-West Region of Cameroon. Forty-two albino Wistar rats aged 3-4 months and weighing 150-200 g were purchased from a farmer in Yaoundé, Centre Region of Cameroon. The reagents and other chemicals utilized were of good purity.

Methods

Extraction and partial purification of African walnut oil Extraction

African walnuts were cracked to remove the shells. The obtained nuts were cut into small pieces and dried in an electric air-drying oven at 50°C for 24 hours. The dried nuts were cold pressed using the HD Manual Oil Press Machine Expeller Extractor Stainless Steel#304 to obtain the oil following the manufacturer's guide. About two kilograms of ground African walnuts were used. The extracted oil was collected in a beaker gradually and later stored in the freezer at -18°C for further analysis and the defatted cake discarded.

Oils degumming

The method reported by Moretto and Fett (1998) was used. In a beaker of 250 mL, 100 g of oil and 3 mL of warm water (70°C) were added. After stirring the mixture for 30 min, it was allowed to decant. The precipitate was removed and the oil filtered and dried using anhydrous sodium sulfate.

Partial Purification of Oils

Purification of oil samples was done following the method described by Ulfah et al. (2023) using palm kernel shell activated carbon and decolorized activated charcoal at 1.5%. Purification was done by adding 100 mL of oil into a 250 mL Erlenmeyer flask. After that, activated carbon was added (1.5%). The Erlenmeyer flask containing the oil and activated carbon was coated with aluminium foil, heated and stirred on a hot plate magnetic stirrer for 60 minutes at 70˚C. A vacuum filter was used to isolate the filtrate. The filtrate was transferred in bottles coated with aluminium foil and kept in the freezer at -18˚C for further analysis.

Formulation of multisource edible oils

Multisource edible oils from palm and African walnut were prepared in two different ratios, 50:50 and 60:40 as recommended by the Food Safety and Standards Authority of India (FSSAI, 2021). The uniform multisource oils were prepared by stirring the sample at 180 rpm (revolutions per minute) for 15 min as reported by Dhyani et al. (2022). It is important to note that saturated oils were warmed at their melting temperatures before use. The samples and their respective blends are presented in Table 1.

Table 1. Composition of different oil samples

Samples	Composition
1	African walnut oil (100%)
2	Palm oil (100%)
3	Palm oil (50%) + African walnut oil (50%)
4	Palm oil (60%) + African walnut oil (40%)

Upon formulation of the multisource oils, all the oils presented in table 1 were initially characterized for their physicochemical properties before being subjected to thermal treatment to study their stability. The parameters analyzed were the colour, peroxide, *p*-Anisidine, TOTOX, acid values and the Fourier-transformed infrared spectroscopy

Oil quality analysis

Colour: The change in colour of oil samples during storage was measured using a FRU WR10 Portable colorimeter. The device was calibrated using an empty petri dish lying on a white surface and the value of the blank recorded. After that, oil was introduced into the petri dish and scanned using the colorimeter. The value for the blank was deducted from the test value and recorded. Analyses were carried out in triplicate. The obtained results were presented as L, a^* and b^*. L* is known as the lightness (L = 0 (black), L = 100 (white); a^* ($-a$ = greenness, $+a$ = redness) and b^* ($-b$ = blueness, $+b$ = yellowness).

Quality indexes: Standard methods were used to analyze oil quality. The peroxide value was determined using the IDF method (IDF, 1991), the p-anisidine and acid values using the AOCS standard method (AOCS, 2003), the thiobarbituric acid value following the method reported by Draper and Hadley (Draper and Hadley, 1990) and the TOTOX value calculated using the following equation as mentioned by Shahidi & Wanasundara (2008): TOTOX = 2PV + AV.

Fourier transformed infrared spectroscopy: The method of Liang et al. (2013) was used for the Fourier transformed infrared spectroscopy of oil samples. A Shimadzu IRPrestige-21 coupled with the DLATGS detector was used. Potassium bromide served as beam splitter and the IRsolution software was used as controller. About 20 µL of sample was dropped between two KBr disks, creating a thin film in the 4000-500 cm^{-1} IR region. The measurements were carried out in duplicate against clean empty KBr disks that served as blank. After each reading, the disks were cleaned twice using hexane, dried with tissue before being washed again with acetone before drying again.

Animal bioassay

Ethical clearance: Animals were cared for and used in agreement with international standard guidelines for animal use. In order to carry out this study, an ethical clearance for animal handling and care was obtained from the University of Buea - Institutional Animal Care and Use Committee (IACUC) with permit UB-IACUC No 02/2024. Animals were under hygienic conditions, they were given water and food ad libitum and their sawdust was changed every two days. They did not feel any pain during the induction of obesity, since it was done by just modifying their diet into a high fat diet. Gavage was done carefully in order not to wound or stress the animals. During the sacrifice too, they were anesthetized so that they are spared from any pains. They were constantly monitored for safety.

Preparation of animal feed: The method described by Othman et al. (2019) with few modifications was used in the formulation of the normal and high fat diets. The normal diet included the following ingredients: 600 g of maize flour, 200 g of soyabeans, 290 g of fish powder, and 10 g of salt for each kilogram of food. The high fat diet (HFD) consisted of 600 g of normal diet, 110 g of boiled egg yolk as source of cholesterol and 290 g of lard, which is a saturated fat from pig. These proportions are for one kilogram of food. After a dough-like consistency was formed using water. The feed was shaped into small balls and dried in the oven at 40^{o} C overnight and used to feed the rats the next morning. Feed was prepared everyday to avoid chemical and biological spoilage.

Treatment of animals: Rats were acclimatized under a 12-hour light-dark cycle at room temperature for 14 days in cages containing sawdust, with ad libitum access to food and water prior to the start of the experiment. A total of forty-two adult male rats was randomly distributed into seven groups of six rats each. Group 2, 3, 4, 5, 6 and 7 were fed with the high-fat diet for 60 days and the oils and Orlistat treatments was done on group 3,4,5,6, and 7 for 28 additional days. The characteristic of each group is presented in Table 2.

After 28 days of treatment, the rats were fasted overnight and anesthetized using Ketamine 60mg/Kg and Diazepam 10mg/kg body weight and the blood collected by cardiac puncture using a 5 mL syringe. The blood was divided into two portions; the first one was introduced in tubes with EDTA and was used for hematological evaluation. The second was introduced in a tube without EDTA and the serum obtained by centrifugation (3000 rpm for 15 min). The serum was used for the evaluation of oxidative stress and inflammatory cytokines. Organs of interest (heart, kidney, pancreas, brain, spleen and liver) were collected and weighed. They were used to prepare organs homogenates (22 g of organ/100 mL of distilled water) which were centrifuged at 3000 rpm for 10 min and used for the determination of oxidative stress markers.

Determination of hematological parameters: Hematological analyses were carried-out on blood samples introduced in tubes containing EDTA. They were done using an automatic hematological analyzer (SFRI H18 LIGHT auto Hematology Analyzer). The parameters analysed were: White blood cells (WBC), Red blood cells (RBC), Hemoglobin (HGC), MID (Mid-size white blood cells), Haematocrit (HCT), Mean copuscular volume (MCV), Granulocytes (GRAN), Platelet (PLT), Mean Platelet Volume (MPV), Platelet Large Cell Ratio (PLCR), Platelet Distribution Width (PDW), Mean Corpuscular Haemoglobin (MCH) and MCHC.

Analysis of some inflammatory cytokines: Serum was used for the analysis of inflammatory cytokines. TNF-α, INF-γ, IL-1β, and IL-6 were quantified using an ELISA kit (Quantikine Colorimetric ELISA Kits (Quantikine®), following instructions provided by the manufacturer. The ELISA kits were obtained from R&D Systems Biotechne, USA.

Determination of oxidative stress parameters: Serum and organ homogenates were used for the measurement of oxidative stress parameters.

Malondialdehyde (MDA)

Malondialdehyde (MDA) level was measured following the method described by Yagi (1976). About 100 µl of serum/ organ homogenate was introduced into test tubes followed by 500 µL of 1% thiobarbituric acid (prepared in 1% trichloroacetic acid) and 500 µL of 1% phosphoric acid. The mixture incubated at 100°C for 15 min and then allowed to cool for 30 min. The tubes were later centrifuged at 3000 rpm for 10 min and the supernatant collected. The absorbance of the supernatant was recorded at 532 nm. The blank was prepared under similar conditions but the sample was replaced by distilled water.

The MDA concentration was calculated using the extinction coefficient 1.56 × 105 $M^{-1}cm^{-1}$ and expressed as µmol of MDA per g of tissue following the formula:

$$[\text{MDA}]\ (\mu\text{mol/g tissue}) = \frac{A}{E \times L}$$

Where; A= Absorbance, Vt=the total volume of the medium (ml), V1=volume of sample (in cuvette); m = weight of tissue used (g); L = light path = 1cm; E= Extinction coefficient =1.56 × 105 $M^{-1}cm^{-1}$

Table 2. Animal group distribution and characteristics

Groups	Characteristics
Group 1 (normal)	Healthy rats fed with the normal rodent chow
Group 2 (negative control)	Obese rats + distilled water (250 mg/kg bodyweight (BW)
Group 3 (positive control)	Obese rats + oral administration of Orlistat (10 mg/ kg) for 28 days
Group 4 (test group 1)	Obese rats + oral administration of 1000 mg/ kg of African walnut oil for 28 days by gavaging
Group 5 (test group 2)	Obese rats + oral administration of 1000 mg/ kg of palm oil oil for 28 days by gavaging
Group 6 (test group 3)	Obese rats + oral administration of 1000 mg/ kg of a 50:50 (PO:WO) multisource edible oil from palm and African walnut oils for 28 days by gavaging
Group 7 (test group 4)	Obese rats + oral administration of 1000 mg/ kg of a 60:40 (PO:WO) multisource edible oil from palm and African walnut oils for 28 days by gavaging

Reduced glutathione (GSH)

Reduced glutathione (GSH) level in organ homogenates and serum was assayed as described by Ellman (1959). About 100 µL of organ homogenates or serum was added to test tubes followed by 900 µL of Ellman reagent (0.4 mg/mL) (prepared in tris-HCl buffer (0.1M, pH 6.5). After stirring the tubes using a vortex, the mixture was incubated at room temperature for 30 min. The absorbance was recorded at 412 nm against a blank which contained 900 µL of the reactive solution and 100 µL of NaCl 0.9% and incubated under the same conditions. The concentration of Thiol groups (SH) was measured by the extinction coefficient (Ɛ) of 13600 $M^{-1}cm^{-1}$ and expressed as µmol/g of total protein. The concentration of GSH was determined as follow:

$$A=Ɛ\,L$$

Where; A = absorbance, L = path length in centimetres, C = concentration in Moles/litter (M), Ɛ the extinction coefficient

Catalase activity (CAT)

The method used by Sinha (1972) was applied for the determination of the catalase activity. About 750 µL of phosphate buffer (pH 7.4, 0.1M) was introduced into a test tube, followed by the addition of 50 µL of sample (organ homogenate or serum). The reaction was initiated by the addition of 100 µL of H_2O_2 (50mM) and a minute after, 1 ml of potassium dichromate (5%) which was prepared in 1% acetic acid. The tubes were then incubated at 100 ˚C for 10 min and the absorbance recorded at 570 nm against a blank (50 µ NaCl 0.9%) prepared under similar conditions. The Catalase activity, expressed as µmol H_2O_2 used/min/mg of protein was determined using the formula:

$$\text{Catalase activity} = Ab \times 1000 \times Vt \times Ɛ \times V$$

Where: Ab: mean of absorbances, **Ɛ**: molar absorbtion coefficient. (40 M cm^{-1}); V: volume of organ homogenate or serum; Vt: Total volume of reaction medium

Nitric oxide (NO) level

The nitric oxide (NO) level determined following the method reported by Montgomery and Doymock (1961). About 100 µL of organ homogenate/serum was mixed with 100 µL of sulfanilamide 1% (prepared in phosphoric acid 5%) followed by incubation at room temperature for 5 min. After that, 100 µL of 0.1% Naphtylethelene diamine (NED) was added and the mixture was incubated once more at room temperature for 5 min. Formation of a purple magenta was indicative of the presence of nitrite formed. The absorbance was recorded at 540 nm. The concentration of NO was determined from the standard curve using different concentrations of $NaNO^2$. NO concentration was determined using its molecular coefficient of **Ɛ**=39500$M^{-1}.cm^{-1}$and calculated as thus:

$$\text{NO Concentration (µM/L)} = \frac{\text{Absorbance}}{Ɛ \times L}$$

Statistical Analysis

The obtained data (Mean ± Standard deviation) were subjected to one-way analysis of variance (ANOVA) using Statgraphics Centurion version XVI in order to evaluate the statistical significance of the data. A probability value at $p<0.05$ was used for statistical significance.

Results

Oil Quality

The initial physicochemical properties of oil samples used in this study are presented in Table 3. The colour of PO was found to be more black, more red and more yellow (*L*, *a**, *b** respectively); WO was more black, normal and more yellow; 50:50 (PO:WO) more black, more red, more yellow and 60:40 (PO:WO) more black, more red and more yellow. The peroxide, p-anisidine, TOTOX, acid and iodine values were respectively between 2.52-3.87meq O_2/Kg, 8.24-12.33, 13.37-19.46, 0.32-20.34 mg KOH/g and 55.21-166.41 g I_2/100g. The highest iodine value was recorded with 100%WO followed by 50:50 (PO: WO), 60:40 (PO: WO) and 100%PO respectively.

The Fourier Transform Infrared Spectroscopy of oil samples are presented in Figure 1 (A-D). The results revealed no significant ($p > 0.05$) peak between 3250 and 3750 cm^{-1}. On all the 4 figures, a peak was recorded at 3000 cm^{-1}, and another peak at 2900 cm^{-1}, an abundant peak was recorded at 1750 cm^{-1}. No peak was found at 2750 cm^{-1} and 1600 cm^{-1}. Looking at the fingerprint region, a peak was recorded around 900 cm^{-1} with the highest intensity found in100%PO. Another peak was found at 750 cm^{-1}.

Table 3. Characterization of the oils physicochemical properties

Parameter		Palm oil (PO) (100%)	Walnut oil (WO)(100%)	50:50 (PO:WO)	60:40 (PO:WO)	Standard CSX 329-2017 (WHO/FAO)
Colour	*L**	-30.13±0.00^a (More black)	-27.15±0.94^a (More black)	-28.52±0.04^a (More black)	-29.41±0.26^a (More black)	/
	*a**	*4.075±0.00^a (More red)	*-0.58±0.00^b (Normal)	*4.55±0.31^a (More red)	*2.40±0.04^c (More red)	/
	*b**	*7.27±0.00^a (More yellow)	*4.52±0.82^b (More yellow)	*9.01±0.31^a (More yellow)	*8.72±0.33^a (More yellow)	/
Peroxide value (meq O_2/Kg)		3.16±0.21^a	2.52±0.00^a	3.87±0.00^a	2.56±0.00^a	≤15 meq O_2/Kg
p-Anisidine value		9.39±0.07^a	12.33±0.00^b	11.72±0.00^b	8.24±0.00^a	≤20 in fish oil
TOTOX value		15.72±2.51^{ac}	16.83±0.00^a	19.46±0.00^b	13.37±0.00^c	≤26 in fish oil
Acid value (mg KOH/g)		0.32±0.00^a	20.34±0.22^b	12.17±1.35^c	10.25±0.45^c	4 mg KOH/g
Iodine value (gI_2/100 g)		55.21±1.24^a	166.41±3.54^b	97.45±1.32^c	85.41±3.42^d	/

n=3 values are presented as mean±SD. $^{a-d}$Values with different superscript letters in the same row are significantly different at $p<0.05$ indicating significance. WO= African walnut oil; PO= Palm oil; 50:50 (PO:WO)= 50:50 (Palm oil : African walnut oil); 60:40 (PO:WO)= 60:40 (Palm oil : African walnut oil).

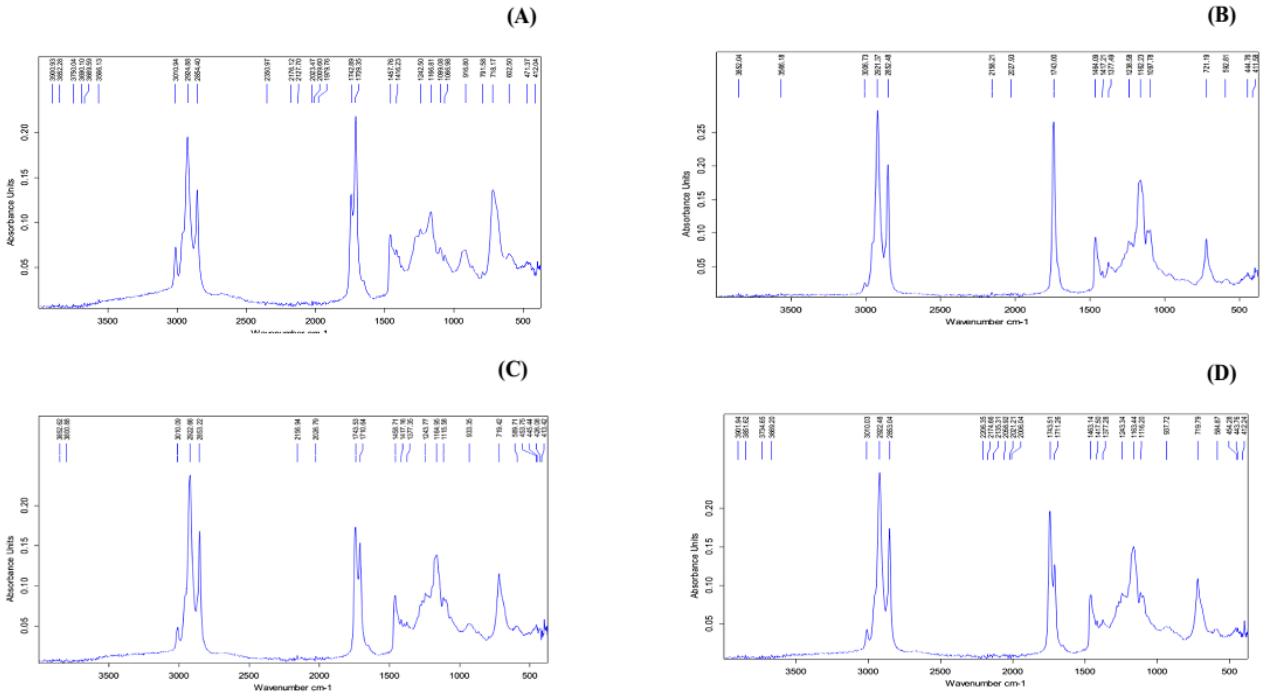

Figure 1 (A-D): Fourier Transfer Infrared Spectroscopy of A: African walnut oil; B: Palm oil; C: 50:50 (Palm oil: African walnut oil); D: 60:40 (Palm oil: African walnut oil)

Table 4. Effect of African walnut oil, palm oil and their blends on Hematological parameters in HFD obesity-induced Wistar rats

	WBC ($\times10^9$/L)	LYM (%)	MID (%)	GRAN (%)	RBC ($\times10^{12}$/L)	HGB (g/dL)	HCT (%)	MCV (fL)
Normal	2.90±0.72^{a}	66.00±3.45^{a}	16.87±1.25^{a}	18.46±2.67^{a}	7.29±0.82^{a}	14.36±0.98^{a}	54.86±2.08^{a}	54.86±1.36^{ab}
Negative control	3.00±0.14^{ab}	61.46±7.01^{a}	19.53±1.24^{a}	22.10±3.25^{a}	7.51±1.16^{a}	14.07±1.60^{a}	43.86±4.09^{b}	56.35±2.16^{a}
Orlistat (10 mg/kg)	5.46±1.49^{b}	66.95±7.24^{a}	18.72±2.29^{a}	21.36±0.55^{a}	7.65±0.44^{a}	13.16±0.98^{a}	39.50±2.48^{c}	51.70±1.52^{b}
100% WO[1]	5.32±1.10^{b}	61.90±4.71^{a}	23.67±3.21^{ab}	20.86±3.20^{a}	7.82±0.77^{a}	13.65±1.61^{a}	41.22±3.96^{b}	52.60±1.56^{ab}
100% PO[1]	4.56±0.55^{b}	51.8±10.01^{a}	26.23±4.78^{b}	25.96±3.00^{a}	8.05±0.53^{a}	13.30±2.10^{a}	42.02±4.00^{b}	52.71±1.70^{ab}
50:50 (PO: WO)[1]	4.95±0.77^{b}	81.65±3.04^{a}	11.70±1.27^{c}	6.65±1.76^{b}	7.46±0.45^{a}	12.50±0.95^{a}	38.40±2.16^{c}	51.56±0.95^{ab}
60:40 (PO: WO)[1]	10.75±3.32^{c}	65.06±5.32^{a}	18.63±2.40^{a}	16.30±3.76^{c}	8.31±0.43^{a}	14.07±0.35^{a}	42.30±0.88^{b}	51.15±1.81^{ab}
	MCH (pg)	MCHC (g/dL)	PLT (10^9/L)	MPV (fL)	PCT (%)	P-LCR (%)	PDW (fL)	
Normal	17.86±1.22^{a}	32.64±1.72^{a}	829.66±104.88^{a}	6.48±0.23^{a}	0.546±0.08^{a}	8.27±1.03^{a}	8.42±0.26^{a}	
Negative control	17.52±1.06^{a}	31.84±3.35^{a}	734.50±52.84^{ab}	6.04±0.30^{a}	0.42±0.18^{a}	5.66±1.12^{b}	7.84±0.99^{a}	
Orlistat (10 mg/kg)	17.15±0.70^{a}	33.25±0.68^{a}	774.00±78.31^{ac}	6.31±0.24^{a}	0.48±0.05^{a}	8.15±1.96^{a}	8.46±0.58^{a}	
100% WO[1]	16.63±1.34^{a}	31.73±2.13^{a}	679.00±0.00^{d}	6.30±0.04^{a}	0.04±0.00^{a}	7.52±0.70^{a}	8.21±0.93^{a}	
100% PO[1]	17.03±1.07^{a}	32.41±1.40^{a}	715.33±27.20^{ac}	6.40±0.24^{a}	0.48±0.07^{a}	8.03±2.19^{a}	8.30±0.18^{a}	
50:50 (PO: WO)[1]	16.70±0.52^{a}	32.46±0.68^{a}	807.00±16.82^{a}	6.33±0.20^{a}	0.50±0.02^{a}	7.33±0.20^{a}	8.30±0.30^{a}	
60:40 (PO: WO)[1]	16.90±0.48^{a}	33.12±0.47^{a}	599.00±70.01^{c}	6.40±0.31^{a}	0.36±0.04^{a}	7.82±2.08^{a}	8.15±0.51^{a}	

1: (1000 mg/kg), n=6 values are presented as mean±SD. $^{a-d}$Values with different superscript letters in the same column are significantly different at $p<0.05$ indicating significance. White blood cells (WBC), Lymphocytes (LYM), granulocytes (GRAN), red blood cells (RBC), Haemoglobin (HGB), Haematocrit (HCT), (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets (PLT), Mean platelet volume (MPV), platelet count (PCT), Patelet larger cell ratio (PLCR), Platelet distribution width(PDW). Palm oil (PO), African walnut oil (WO) and PO:WO (50:50) and PO:WO(60:40).

Hematological Parameters

The effect of oils on the hematological parameters of rats is presented in Table 4. No significant ($p > 0.05$) difference was observed between the WBC of the normal and negative control. However, the WBC levels were significantly higher ($p<0.05$) in rats treated with Orlistat and oils. For the lymphocytes, no significant difference was recorded between the LYM% of all groups except for the rats treated with 50:50 (PO:WO) which was significantly higher ($p < 0.05$). The rats fed with 100%PO presented the highest MID compared to the other groups, while the lowest value was recorded in rats fed with 50:50 (PO:WO). 50:50 (PO:WO) and 60:40(PO:W0) significantly ($p < 0.05$) decreased the GRAN concentration in the blood of rats compared to the other groups. No significant difference ($p > 0.05$) was recorded in the values for RBC, HGB, MCV, MCH, MCHC, MPV, PLT, PCT, P-LCR, PDW across the groups. The concentration of HCT significantly reduced in the rats fed with the HFD compared to the normal group. Similar observations were made with PLT number.

Inflammatory Cytokines

Results for inflammatory cytokine are presented on Figure 2 (A-D). Serum levels of TNF-α, INF- γ, IL-1β and IL-6 were significantly ($p<0.05$) higher in negative control group as compared to the normal group, Orlistat, 100% WO, 100%PO, 50:50 (PO:WO), and 60:40 (PO:WO).

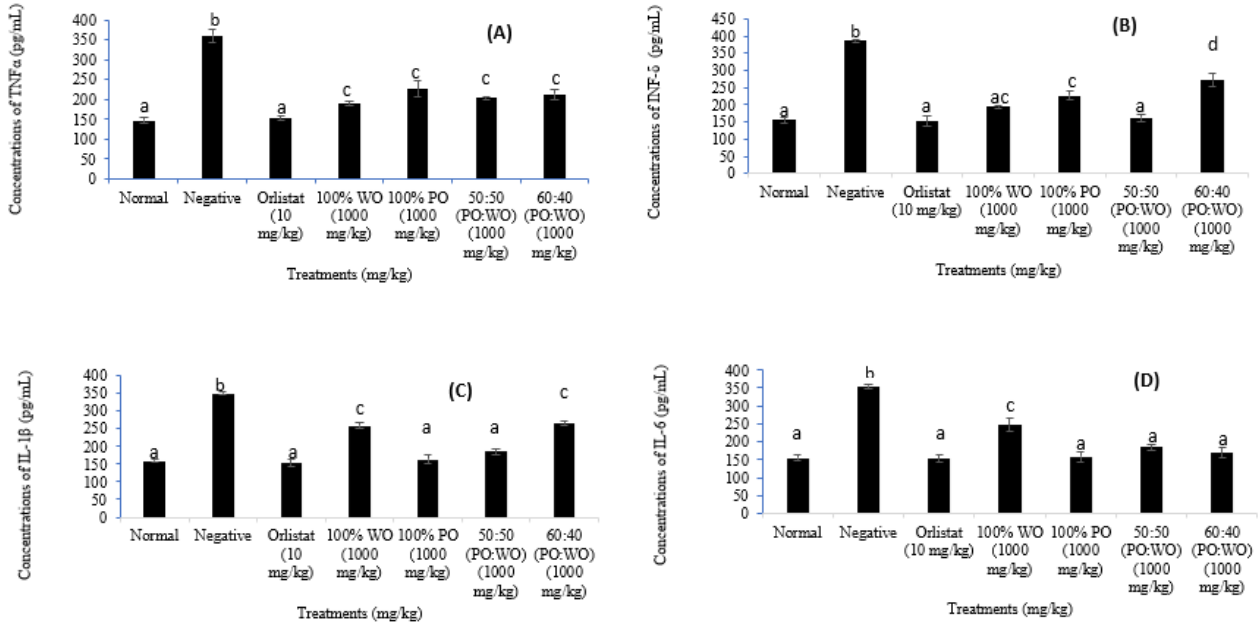

Figure 2. (A-D): Inflammatory cytokine: TNF-α (A), INF-δ (B), IL-1β (C) and IL-6 (D) levels in experimental groups
n=6 values are presented as mean ± SEM. Values with different superscripts are significantly difference at ($p<0.05$). PO; Palm oil, WO; African Walnut oil, 50:50 (PO:WO); 50:50 (Palm oil: African walnut oil), 60:40 (PO:WO); 60:40 (Palm oil: African walnut oil), TNF-α; Tumor necrotic factor-α, INF- γ; Interferon-δ, IL-1β; Interleukin- 1β and IL-6; Interleukin-6

Table 5. Effect of African walnut oil, palm oil and their corresponding blends on CAT levels in organ homogenate and serum (µmol H_2O_2/min/mg

	Spleen	Kidney	Heart	Brain	Pancreas	Liver	Serum
Normal	10.92±1.10^{a}	11.49±0.46^{a}	15.12±1.01^{a}	18.48±0.00^{a}	14.10±0.00^{a}	17.16±0.00^{a}	20.37±2.50^{a}
Negative control	10.80±0.00^{a}	9.90±0.42^{a}	14.70±1.78^{a}	16.56±4.32^{ab}	12.69±1.90^{ab}	20.01±0.04^{b}	18.92±1.41^{ab}
Orlistat (10 mg/kg)	9.82±0.24^{a}	11.08±2.06^{a}	15.86±1.17^{a}	17.62±1.86^{a}	10.20±0.33^{b}	17.19±3.86^{ab}	20.72±1.78^{a}
100% WO[1]	12.62±1.02^{ab}	11.67±1.14^{a}	13.00±0.29^{a}	21.66±1.27^{b}	11.32±1.05^{b}	15.09±1.82^{ac}	14.32±2.43^{b}
100% PO[1]	12.63±0.63^{ab}	10.36±0.78^{a}	13.74±1.56^{a}	17.72±1.88^{a}	10.56±2.12^{ab}	13.11±1.14^{c}	22.90±2.82^{a}
50:50 (PO: WO)[1]	10.44±0.00^{a}	11.04±0.00^{a}	15.06±0.00^{a}	20.94±0.00^{ab}	12.06±0.00^{ab}	17.88±0.00^{ab}	22.26±5.15^{a}
60:40 (PO: WO)[1]	13.68±0.00^{b}	9.00±0.00^{a}	14.79±1.23^{a}	22.29±1.48^{ab}	13.44±1.78^{ab}	16.86±0.00^{abc}	22.44±3.49^{a}

1: (1000 mg/kg), n=6 values are presented as mean±SD. a-cValues with different superscript letters in the same column are significantly different at $p<0.05$ indicating significance. Palm oil (PO), African walnut oil (WO) and PO:WO (50:50) and PO:WO(60:40).

Table 6. Effect of African walnut oil, palm oil and their corresponding blends on GSH levels in organ homogenate and serum (µmol)

	Spleen	Kidney	Heart	Brain	Pancreas	Liver	Serum
Normal	40.07±0.00^{a}	117.50±25.37^{a}	142.27±7.27^{ab}	64.33±0.00^{a}	60.07±0.00^{a}	129.08±0.67^{ac}	45.58±0.51^{a}
Negative control	126.02±0.00^{b}	112.31±18.04^{a}	134.55±12.16^{ab}	104.55±0.00^{bc}	27.94±14.14^{bc}	132.79±0.00^{a}	67.94±10.08^{b}
Orlistat (10 mg/kg)	24.41±0.00^{c}	110.77±2.85^{a}	141.37±3.63^{ab}	75.18±23.24^{ab}	37.23±18.54^{b}	139.70±1.08^{b}	97.53±10.76^{c}
100% WO[1]	62.38±10.24^{d}	112.67±20.67^{a}	124.60±20.87^{ab}	115.51±6.23^{c}	21.94±8.57^{bc}	127.89±17.46^{abc}	59.74±1.81^{b}
100% PO[1]	141.43±7.64^{e}	101.91±12.45^{a}	116.88±20.64^{a}	73.757.27^{ab}	13.63±1.2^{c}	133.65±7.19^{ab}	52.27±14.97^{b}
50:50 (PO: WO)[1]	97.05±0.00^{f}	102.20±0.00^{a}	134.26±0.00^{b}	105.73±0.00^{b}	68.45±0.00^{a}	133.75±0.00^{a}	45.33±1.50^{a}
60:40 (PO: WO)[1]	57.64±0.006^{d}	127.42±6.13^{a}	133.52±0.18^{b}	122.09±4.10^{b}	39.26±0.00^{b}	117.86±5.40^{c}	90.34±8.51^{c}

1: (1000 mg/kg), n=6 values are presented as mean±SD. $^{a-d}$Values with different superscript letters in the same column are significantly different at $p<0.05$ indicating significance. Palm oil (PO), African walnut oil (WO) and PO:WO (50:50) and PO:WO (60:40).

Effect on Oxidative Stress Parameters

Effect of Oils on CAT Activity in Organ Homogenate and Serum

The catalase activity of organ homogenates and serum of normal and HFD rats is presented in Table 5. Generally, no significant ($p > 0.05$) difference in this parameter was observed in the spleen, of the normal and HFD groups except for the rats that received 60:40 (PO:WO) who presented a significantly higher ($p < 0.05$) catalase activity. Similarly, no significant ($p > 0.05$) change in CAT activity was recorded in the kidney and heart of the animals across all groups. At the level of the brain, rats treated with 100% PO, 50:50 (PO:WO) and 60:40 (PO:WO) presented the highest CAT activities compared to the normal group and negative control. At the level of the pancreas, rats that received Orlistat and 100% PO presented significantly ($p < 0.05$) lower CAT activities compared to the rats of the other groups.

Effect of oils on GSH activity in organ homogenate and serum

Table 6 presents the effect of oils and Orlistat on the GSH activity of HFD rats. A significant increase ($p < 0.05$) in this parameter was recorded in the spleen, of the negative control and the rats treated with 100%PO compared to the other groups where GSH activity was significantly ($p < 0.05$) lower. No significant ($p > 0.05$)

difference was recorded in the GSH activity of the kidney homogenates in all groups. At the level of the heart, the GSH activity was higher in the normal group but lower in the rats that received the HFD even though it was only significant in the rats treated with PO:WO (60:40) and PO;WO (50:50). At the level of the brain, a significant increase ($p < 0.05$) in GSH activity was observed in all the groups that received the HFD compared to the normal group. The group treated with Orlistat and 100% PO exhibited the lowest GSH activities compared to the negative control. At the level of the pancreas, the rats of the normal group and those that received PO:WO (50:50) presented the highest GSH activities compared to the other groups. At the level of the liver, the highest GSH activity was obtained with the group treated with Orlistat and the lowest in the one treated with PO:WO (60:40). No significant difference ($p < 0.05$) was observed in this parameter among other groups. Looking at the GSH of the serum, it generally significantly increased ($p > 0.05$) in groups that received the HFD compared to the normal group. The group treated with Orlistat and 60:40 (PO:WO) exhibited the highest GSH levels.

Effect of oils on MDA levels in organ homogenate and serum

The level of malondialdehyde in organ homogenates and serum of rats is presented in Table 7. At the level of the spleen, almost all the rats fed with the HFD presented significantly ($p < 0.05$) higher MDA concentrations compared to the normal group, and the highest value recorded with the negative control group. No significant ($p > 0.05$) difference was observed between the MDA level of the normal group and the rats treated with 100% WO, PO:WO (60:40). At the level of the kidney, the negative control group exhibited significantly ($p < 0.05$) higher MDA levels compared to all the other groups. A significant ($p < 0.05$) increase was seen in the MDA levels in the heart of all the rats that received the HFD compared to the normal group. This parameter was higher in the heart of the rats that received Orlistat or oil, compared to the negative control though this was not significant. At the level of the brain, the rats treated with Orlistat and PO:WO (50:50) presented significantly ($p < 0.05$) lower MDA levels compared to the other groups in which the MDA levels remained similar. For the pancreas, the rats fed with Orlistat, 100%WO and 100%PO plus those of the normal group showed significantly ($p < 0.05$) lower MDA levels compared to the other groups. In the liver, the negative control and the group treated with 100%PO presented the highest MDA concentration compared to the other groups. A similar observation was made at the level of the serum.

Effect of oils on Nitric Oxide (NO) levels in organ homogenate and serum

Table 8 shows the NO levels of HFD rats treated and untreated compared to the normal group. Generally, this parameter increased in the groups treated with oils as well as the normal group compared to the negative control and the group treated with Orlistat. At the level of the spleen, no significant ($p > 0.05$) difference was observed between the NO levels of all rats treated with PO:WO (50:50) in which the NO levels were significantly ($p < 0.05$) higher. At the level of the kidney, the homogenates from rats treated with Orlistat, 100% PO, PO:WO (50:50) showed significantly higher NO levels compared to the other groups. The NO level of the heart was significantly ($p < 0.05$) higher with the normal group, compared to the groups that received the HFD. No significant ($p > 0.05$) difference was recorded between the negative control and the treated groups. The NO levels in the brain of rats that received the HFD and treated were generally significantly ($p < 0.05$) higher compared to that of the negative control. However, the NO level in the group treated with 100% PO was similar to that of the negative control. At the level of the pancreas, the NO level in the normal group was significantly ($p < 0.05$) higher than that of the other groups, the negative control presenting the lowest value. At the level of the liver, the NO level of the negative control was significantly ($p < 0.05$) lower than that of the rat treated with Orlistat and oil but was similar to that of the normal group. At the level of the serum, the normal group presented a significantly ($p < 0.05$) lower NO value, compared to the groups that received the HFD and were treated. However, its value was not different from that of the negative control.

Table 7. Effect of African walnut oil, palm oil and their corresponding blends on MDA levels in organ homogenate and serum (µmol/L)

	Spleen	Kidney	Heart	Brain	Pancreas	Liver	Serum
Normal	1.88±0.69 ^{a}	1.04±0.26 ^{a}	1.83±0.02 ^{a}	2.56±0.60 ^{ab}	1.56±0.00 ^{a}	1.03±0.07 ^{a}	1.86±0.44 ^{a}
Negative control	4.22±0.00 ^{b}	3.27±0.00 ^{b}	2.59±0.47 ^{b}	2.51±0.00 ^{a}	4.59±0.00 ^{b}	2.74±0.30 ^{b}	3.01±0.39 ^{b}
Orlistat (10 mg/kg)	3.07±0.79 ^{b}	1.40±0.00 ^{a}	3.91±0.79 ^{bc}	1.98±0.02 ^{b}	3.20±0.04 ^{c}	1.16±0.00 ^{a}	2.35±0.52 ^{ab}
100% WO[1]	2.08±0.97 ^{a}	2.22±0.07 ^{c}	4.00±0.42 ^{c}	2.25±0.21 ^{a}	1.74±0.11 ^{a}	1.21±0.00 ^{a}	1.85±0.61 ^{c}
100% PO[1]	3.70±0.83 ^{b}	1.00±0.23 ^{a}	3.18±0.00 ^{bc}	2.77±0.14 ^{a}	3.40±0.65 ^{bc}	2.60±0.36 ^{b}	2.42±0.37 ^{ab}
50:50 (PO: WO)[1]	2.97±0.00 ^{b}	2.46±0.00 ^{a}	3.41±0.00 ^{bc}	1.97±0.00 ^{b}	3.85±0.00 ^{bc}	1.58±0.00 ^{a}	2.20±0.33 ^{ab}
60:40 (PO: WO)[1]	2.03±0.00 ^{a}	1.72±0.00 ^{a}	3.58±0.83 ^{bc}	2.30±0.70 ^{a}	4.49±0.10 ^{b}	1.66±0.00 ^{a}	2.71±0.70 ^{ab}

1: (1000 mg/kg), n=6 values are presented as mean±SD. $^{a-c}$Values with different superscript letters in the same column are significantly different at $p<0.05$ indicating significance. Palm oil (PO), African walnut oil (WO) and PO: WO (50:50) and PO:WO(60:40).

Table 8. Effect of African walnut oil, palm oil and their corresponding blends on NO levels in organ homogenate and serum (µM/L)

	Spleen	Kidney	Heart	Brain	Pancreas	Liver	Serum
Normal	16.84±5.3^{ab}	11.11±0.00^{a}	13.72±3.07^{a}	10.81±0.00^{a}	37.73±3.16^{a}	28.34±0.69^{a}	1.31±0.00^{a}
Negative control	8.46±1.41^{a}	12.27±0.00^{a}	8.94±3.63^{ab}	7.40±0.41^{b}	13.46±0.57^{b}	22.45±3.22^{a}	3.15±1.55^{a}
Orlistat (10 mg/kg)	10.27±3.29^{ab}	34.31±2.41^{b}	9.59±2.75^{ab}	12.58±1.57^{a}	22.05±1.67^{c}	36.15±0.75^{b}	6.97±0.91^{b}
100% WO[1]	15.98±2.51^{ab}	9.65±1.05^{a}	6.60±2.62^{b}	18.84±1.19^{c}	29.03±0.00^{d}	35.53±2.63^{b}	11.24±4.18^{b}
100% PO[1]	14.07±3.16^{ab}	33.65±2.16^{b}	6.66±2.80^{b}	7.56±0.75^{b}	24.40±5.33^{cd}	36.35±3.49^{b}	8.37±0.00^{b}
50:50 (PO: WO)[1]	23.59±0.00^{c}	21.51±0.00^{c}	6.70±1.05^{b}	20.37±0.00^{c}	16.73±0.00^{b}	35.39±0.00^{b}	5.54±0.00^{a}
60:40 (PO: WO)[1]	14.44±6.03^{ab}	14.65±0.00^{a}	6.77±1.73^{b}	12.73±2.79^{a}	25.64±0.00^{cd}	35.06±1.93^{b}	11.44±0.00^{b}

1: (1000 mg/kg), n=6 values are presented as mean±SD. $^{a-d}$Values with different superscript letters in the same column are significantly different at $p<0.05$ indicating significance. Palm oil (PO), African walnut oil (WO) and PO:WO (50:50) and PO:WO(60:40).

Discussion

Initial Oil Quality

The colour characteristics of oils can be attributed to various pigments and compounds present in the oil, such as carotenoids and chlorophylls. The L*, a*, b* scale has been adopted for determining the colour of edible oils and on this scale, L* is light/dark with L values from 0-50 representing lightness and L* values from 51-100 representing darkness, a* is red/green with positive a values representing redness and negative a values representing greenness. b* is yellow/blue with positive b values representing yellowness and negative b values representing blueness (Klisic et al., 2023). Results showed that palm oil (PO) exhibited higher values of blackness, redness, and yellowness compared to African walnut oil (WO). This could be attributed to its high content in carotenoids compared to WO. WO had higher blackness and yellowness values but showed a normal level of redness. In the case of palm oil, its characteristic deep red colour is predominantly due to the presence of carotenoids, specifically β-carotene (Tan et al., 2021). The darker colour observed in palm oil compared to walnut oil could be attributed to a higher concentration of these pigments. Walnut oil, on the other hand, contains a lower number of carotenoids (Oloko, 2019) and so it exhibited a normal a* value. The variations in colour observed in the oil -50:50 (PO:WO) and 60:40 (PO:WO) blends, can be attributed to the combined pigments from both palm oil and walnut oil. The addition of palm oil to African walnut oil may contribute to the increased redness and yellowness, resulting in a more intense color profile than with African walnut oil alone.

Peroxide value indicates the extent of primary oxidation in an oil particularly the presence of hydrogen peroxide (Ali et al., 2020). The peroxide values for all oil samples analysed ranged between 2.52 and 3.87 meq O_2/kg which was within the safety range established by *Codex Alimentarius* (≤15 meq O_2/Kg) for crude and virgin edible oils (FAO/WHO, 2009).

P-anisidine value is a measure of secondary oxidation products in oils, marked by the formation of aldehydes, especially 2-alkenals and 2,4-dienals (He and Liu, 2019). The P-anisidine value of all the oil samples analysed ranged from 8.24-12.33 and were within the standard (≤20 in fish oil) established by *Codex Alimentarius*, indicating moderate levels of secondary oxidation in the oils and blends (FAO/WHO, 2009).

TOTOX value measures the overall oxidation state of an oil, combining peroxide and p-anisidine values (Bannenberg et al., 2017). This value better describes the total oxidative state to which a fat or oil has been exposed. Results showed that the TOTOX values obtained in this study ranged from 13.37 to 19.46 which are within the accepted range established by *Codex Alimentarius* (≤26 in fish oil) (FAO/WHO, 2009). The moderate TOTOX values suggest a moderate level of oxidative deterioration in the oils

Acid value is an indicator of oil degradation and it informs on the breakdown of triglycerides into free fatty acids (Sharma and Jain, 2015). The acid value of all the tested oil samples (from 0.32 to 20.34%), was generally higher than 0-4 mg KOH/g which is the recommended range for crude and virgin oils. However, it was lower than 45 mg KOH/g which is the recommended range in fish oils with a high phospholipid concentration of 30 percent or more such as krill oil (FAO/WHO, 2009). The high in acid value found with African walnut oil (20.34 mg KOH/g) suggests its high potential for degradation among the samples.

Iodine value is a measure of the degree of unsaturation of fats and oils. Higher iodine values indicate the presence of multiple double bonds and hence higher levels of unsaturated fatty acids (Geng et al., 2023). The results in this study showed that the iodine values ranged from 55.21 to 166.41 g I_2/100g with the highest iodine value observed in 100% WO, followed by 50:50 (PO: WO), 60:40 (PO:WO), and 100% PO. The higher iodine value of African walnut oil compared to palm oil indicates a higher content of unsaturated fatty acids in walnut oil. This result goes to support the findings of Tchiegang et al. (2007) who revealed that African walnut oil contains a higher proportion of unsaturated fatty acids; 9.48% Oleic acid, 14.41% linoleic acid and 70.39% alpha-linolenic acid. Blending it with palm oil reduced its iodine value indicating the blend has a lesser degree of unsaturation and hence is more stable to oxidation.

Fourier Transform-Infrared Spectroscopy (FT-IR) is a technology for acquiring emission spectra or infrared absorption from solid, liquid or gas samples, as molecules absorb radiation at very specific wavelengths (Kassem et al., 2023). The FTIR spectrum employed for the four samples in this study was measured within the wave numbers between 400 cm^{-1} to 4000 cm^{-1}. Almost all edible oils consist of triacylglycerol (92%), low concentrations of di- and monoacylglycerols (5%), and low levels of other components (Jamwal et al., 2019). Thus, the spectra of these oil samples exhibit numerous similarities among absorbance bands in this study. This region is similar to the region chosen by other researchers (Fridah, 2015). The spectral analysis revealed several important peaks that provide insights into the chemical composition of the oils. The results for all oil samples showed there was an absence of peaks between 1600 cm^{-1} - 2750 cm^{-1} and 3250 cm^{-1} - 3750 cm^{-1}, indicating the absence of Aldehydes (C=O), Aldehydes (C-H), and Hydrogen peroxide vibrations respectively, in this spectral region (Poiana et al., 2015). This showed the oils were free of primary and secondary oxidation products. In all oils, a peak was consistently recorded at 3000 cm^{-1}, which corresponds to the stretching vibrations of C-H bonds in the oil samples (Poiana et al., 2015). This peak suggests the presence of hydrocarbon chains in both palm oil (PO) and African walnut oil (WO). This result is not uncommon as it a common functional group found in oils. Another prominent peak observed in the spectra was located at 1750 cm^{-1}, which represents the stretching vibration of the carbonyl (C=O) group, indicating the presence of fatty acid esters or other carbonyl-containing compounds in the oil samples analyzed (Wang et al., 2024). This proved oil quality, indicating that the fatty acids are still linked to glycerol. Furthermore, peaks were found at the wave number 2900 cm^{-1}; indicating the presence of methylene/ methyl groups

which are mostly found in hydrocarbons, and at 3000 cm^{-1}; indicating the presence of a cis bond (C-H) (Poiana et al., 2015). These results revealed that all the samples analysed had some degree of saturation and unsaturation respectively. In the fingerprint region, a peak was recorded around 900 cm^{-1}, with the highest intensity found in 100% palm oil (PO). This peak indicates the presence of isolated trans bonds (C=C-H bend) (Poiana et al., 2015) and hence indicates the presence of unsaturated compounds in this oils. Additionally, another peak at 750 cm^{-1} was observed in the FTIR spectra. This peak corresponds to CH_2 rocking vibrations present in the oil samples (Poiana et al., 2015), supporting the previous vibrations in the functional group zone that suggested the presence of unsaturated compounds in the oils.

Hematology

The blood count is the primary biological test used to screen for hemopathies (Lounguaing et al., 2022). White blood cells (WBC) source out, attack and destroy disease causing microorganisms on a daily basis and therefore play a crucial role in the body's immune response. Results showed no significant ($p>0.05$) difference in the WBC and its sub types in the normal and negative control groups but an increase in the groups treated with orlistat, and the oils. There was an important increase ($p<0.05$) in the LYM % of the rats treated with 50:50 (PO:WO), and in the MID of rats treated with 100% PO compared to the other groups. This increase in white blood cell count could indicate that consumption of orlistat or the various oils in their corresponding groups did not have a negative impact on the defensive function of WBC (Babalola et al., 2016). These results are contrary to the findings of Gu et al. (2018) who concluded that the presence of elevated WBC in obesity is a predisposing factor to developing type 2 diabetes. Granulocytes (GRAN) on the other hand are a type of white blood cells which comprise of neutrophils, eosinophils, and basophils and play an important part in innate immune response and fighting against infections (Gigon et al., 2021). Results found that, treatment with 50:50 (PO:WO) and 60:40 (PO:WO) significantly decreased the GRAN concentration in the blood of rats in this group compared to the other groups. This suggests a reduced need for protection against infection or inflammation in these groups and hence efficacy of the corresponding oil blends in combating the latter.

The absence of variations in RBC, HGB, MCV, MCH, MCHC, MPV, PLT, PCT, P-LCR, PDW suggests that none of the feeding methods, positive control drug (orlistat) or lipid sources used induced anaemia in the experimental rats since RBC, HGB and PCV concentrations are basic values indicating the degree of anaemia (Tirado, 2023). These results were not different from the findings of Babalola et al. (2016) who made similar conclusions from their findings.

Results revealed that the HFD groups exhibited significantly ($p<0.05$) lower values for HCT and PLT compared to the normal group. Similar results were obtained by Adullah et al. (2018). The decrease in platelet number in the high fat diet groups could be due to free radical damage to the stem cells which are the site of production of platelets (Loungaing et al., 2022). However, these findings contradict those of Saputri et al. (2023) who attributed HFD to enhanced PLT activation.

Inflammatory Cytokines

Recent research has shown the link between High fat diet consumption and low-grade inflammation (Moghbeli et al., 2020). Results showed that the negative control group which received the high fat diet alone with no treatment exhibited the highest levels of inflammatory cytokines. This can be explained by the fact that, persistent ingestion of a high fat diet causes changes in the gut microbiota. This leads to increased production of lipopolysaccharides (LPS) which increases the production of inflammatory cytokines (Thomas et al., 2022). This finding can help agree that the rats of the negative control group were obese since obesity elevates the number of blood cytokines such as TNF-α, IL-1β, IL-8 and IL-6 in the circulation (Ashraf et al., 2018). This work was in accordance with a similar study carried out by Schmidt et al. (2015) where there were significant elevations of INF-γ and TNF-α in case of obesity. The results obtained in this study showed that using the oils as an intervention for inflammation in high fat diet induced obesity improved the inflammatory conditions by lowering pro-inflammatory cytokine levels. Interestingly, African walnut oil exerted better improvement effects than palm oil, as evident from the significantly reduced TNF-α and INF-γ levels. This can be attributed to the anti-inflammatory nature of omega-3 rich oils (Guo et al., 2023). African walnut oil is rich in α-linolenic acid (about 69.90%) (Ghomdim et al., 2024). These fatty acids might be responsible for the observed anti-inflammatory activity. Giacobbe et al. (2020) reported that omega-3 fatty acids have the ability to reduce symptoms and have anti-inflammatory property by producing omega-3 fatty acids metabolites such as maresins, revolvins and protectins, which are promediators that act as strong anti-inflammatory agents.

Palm oil on the other hand had better anti-inflammatory effects in reducing the levels of IL-1β and IL-6. This can be attributed to its high content of tocotrienols (a type of vitamin E), which act as a potential antioxidant, and which equally exhibit anti-inflammatory activities (Dauqan et al., 2012; Tan et al., 2020). Wu et al. (2008) demonstrated that tocotrienol-rich fraction of palm oil exerts good anti-inflammatory property by suppressing the expression of inflammatory mediators in human monocytic cells. These results are similar to the findings of Ajuwon et al. (2022) where red palm oil reduced the levels of TNF-α, IL-1β and IL-6. Contrary to these results were the findings of Martins et al. (2024) where consumption of palm oil led to higher plasma levels of IL-6. This could be due to the fact that the palm oil used in this study was inter-esterified palm oil.

As to what concerns the oil blends, interestingly PO:WO (50:50) had better effects against inflammation as it significantly reduced TNF- alpha, INF-γ and IL-1β levels in serum. This can be due to the fact that a diet containing a low ratio of saturated to unsaturated fatty acids is more effective in reducing inflammation due to a higher proportion of anti-inflammatory omega-3 PUFA's, as revealed in the findings of Thomas et al. (2022) who obtained similar results.

Oxidative Stress

Reactive oxygen species have been found to be involved in the control of body weight by exerting different effects on hypothalamic neurons, which control satiety and hunger behaviour (Drougard et al., 2015). HFD induced obesity is associated with mitochondrial dysfunction and increased oxidative stress. It has been reported that mitochondria are the main sources of reactive oxygen species in mammalian cells. Their production leads to mitochondrial damage in a range of pathologies amongst which obesity (Andreyev et al., 2005; Balaban et al., 2005). Research has shown that in excessive adiposity, reactive oxygen species levels increase and exceed the capacity of the antioxidant system (Balan et al., 2024). A protective effect against oxidative stress is seen from an oil's ability to reduce lipid peroxidation and increase anti-oxidant enzyme activity (Dauqan et al., 2012). For example, Omega-3 fatty acids were reported to have antioxidant and anti-inflammatory properties. They are efficient in reducing oxidative stress and inflammation in macrophages from patients with small abdominal aortic aneurysms (Rocha et al., 2022). Heshmati et al. (2019) reported that omega-3 fatty acid supplementation improves antioxidant, glutathione peroxidase and malondialdehyde activities. Thus, omega-3 fatty acids and omega-3 fatty acids-rich oils can be mentioned as enhancer elements in antioxidant activity against Reactive oxygen species. This can help improve the pathological status of many diseases.

In this study, lipid peroxidation examined by organ/serum levels of MDA was shown to be significantly ($p<0.05$) higher in the negative control group than the normal group. Results showed that, the greatest extent of lipid peroxidation was observed in the spleen, kidney, pancreas, liver, and serum of the negative control. This could be attributed to the fact that this group was not treated and receive just the high fat diet throughout the experimental period. Multiple studies have shown that obese patients have higher MDA levels than non-obese patients (Yesilbursa et al., 2005; Huang et al., 2023). This result was also consistent with available information in the literature which noted that increase lipid peroxidation occurs in response to overwhelming rise in ROS (Ichipi-Ifuor et al., 2022). Results revealed that the liver exhibited the highest CAT activity and MDA level in the negative control group. This can be explained by the fact that obesity is characterised by a fatty liver resulting in a higher-than-normal liver weight in a condition referred to as non-alcoholic fatty liver disease (NAFLD). NAFLD is characterized by fat accumulation in the liver, which can lead to increased lipid peroxidation. It is a metabolic disease that leads to fat accumulation in the liver, a condition known as hepatic steatosis. This can appear if the liver cannot properly process fats or if a patient has other conditions like obesity, diabetes, or high triglycerides (Martín-Fernández et al., 2022). The increase CAT activity as shown in this study is an effort to protect the liver from the harmful effects of the reactive oxygen species resulting from this lipid peroxidation (Nandi et al., 2019). Results of this study also found that the heart and brain showed significantly lower MDA levels, with a significantly higher GSH activity in the negative control group. This reflects an effort to protect the cells of the heart against oxidative damage and can be explained by the fact that the glutathione (GSH) system, has been proven to be one of the most powerful endogenous antioxidant systems in the cardiovascular system due to its key contribution to detoxifying xenobiotics (Tan et al., 2023). Similar results were obtained by Meral (2022) who revealed that the low level of MDA in the brain of obese rats indicates the presence of a protective mechanism against hypoxia since the enlargement of adipocytes leads to local hypoxia (Dos Santos Cardoso, 2013).

Looking at the group of rats that received 100%WO, results showed that lipid peroxidation products (MDA) are decreased upon treatment with African walnut oil with a corresponding decrease in GSH and catalase activities in most tissues/ serum. This can be explained by the fact that omega-3 fatty acid rich nature of African walnut oil is able to counteract oxidative stress and lipid peroxidation due to its antioxidant capacity (Rocha et al., 2022; Heshmati et al., 2019). These results are similar to those obtained by Uti et al. (2019) and Abam et al. (2013) who showed that lipid peroxidation in the liver, kidney and brain tissues of rats treated with cadmium was successfully attenuated upon administration of African walnut oil at 2.0 g/kg body weight. In the group treated with PO (100), results showed a decrease in lipid peroxidation in most organs and serum. This effect of palm oil can be related to the β-carotene present that has the ability to quench free radicals and prevent tissue damage, and also due to the fact that vitamin E present in palm oil inhibits the production of lipid hydroperoxides (Dauqan et al., 2012). At the level of the brain the group treated with 100% PO exhibited the lowest GSH activity, and highest CAT activity respectively. Also, the MDA levels were high in the liver and serum of rats in 100% PO. This work is in agreement to the findings of Ichi-Ifukor et al. (2022) who showed that crude palm oil reduced GSH levels in the brain, has no significant difference in CAT activity of the brain but reduces serum and liver levels of MDA in rats exposed to oxidative stress by acute cadmium poisoning.

As to what concerns the blends, the results obtained showed that the multisource oil blends PO: WO (60:40) exhibited high GSH activity in the serum, and high CAT activity in the spleen brain and serum. Also, PO: WO (50:50) showed low levels of MDA at the level of the brain, with high CAT activity in the brain and high GSH activity at the level of the pancreas. Oxidative stress is characterized by depletion in antioxidants/antioxidant enzymes and a high level of lipid peroxidation (Oyem et al., 2021). The results obtained from the administration of this multisource oil are indicative of the fact that the oils had better effects in fighting the adverse effect of the high fat diet by improving the animals' oxidative state and lipid peroxidation, owing to the combined potentials from the omega-3 rich PUFAs African walnut oil (omega-3 fatty acids) (Rocha et al., 2022; Heshmati et al., 2019) and the antioxidant packed palm oil (Vitamin E, β-caroten) (Dauqan et al., 2012).

Nitric Oxide (NO) serves as an important marker of oxidative stress. This study showed that Obesity induction caused significant decrease in NO levels in rats of the negative control group. This can be due to the fact that High fat diet impairs nitric oxide bioavailability (Razni et al., 2011). Eccleston et al. (2011) reported that chronic exposure to a HFD leads to hepatic steatosis, impairs nitric

oxide bioavailability, and modifies the mitochondrial proteome in mice. It can further be explained from the quenching of NO by its action with resulting free radicals, loss of endothelial NO synthase (eNOS) activity or downregulation of eNOS (Sansbuty & Hill, 2014). Similar results were obtained by Niwanthi et al. (2016) who explained that reduced NO availability as seen in high fat diet consumption is caused by impaired transport of its substrate, L-arginine. Results showed that treatment with 100%WO improved and upregulated the NO levels of rats in this group at a similar level to that of normal and orlistat groups, all higher than NO levels in the negative control group. These treatments raised the NO levels in the spleen, brain, pancreas, and liver, with a marked increase in the serum. Although the mechanism of action was not exploited in this study, the results obtained by Niwanthi et al. (2016) permits to suggest that African walnut oil may be involved in the augmentation of endothelial L-arginine transport which results in the production of more NO. Concerning the NO concentration in 100% PO group, all organs and serum of rats in this group had high NO levels when compared to the negative control group except for the heart. There was a significant increase in NO at the level of the kidney and the liver. The significantly higher NO levels seen in the kidney can be due to the fact that fresh palm oil supports glomerular flow rate, renal plasma flow (Beshel et al., 2014). It can also be due to the fact that NO is involved in modulation of renal autoregulation as well as tubular fluid and electrolyte transport (Carlstrom, 2021). The high NO levels revealed in all groups might be attributed to the fact that NO maintains liver homeostasis and inhibits the pathological conditions of the liver. The increase is contrary to the findings of Albrahim et al. (2022) who showed low levels of MDA results from the administration of red palm oil to rats fed a hypercholesterolemic diet.

Conclusion

The objective of this work was to evaluate the effects of palm oil, African walnut oil and their blends (50:50 and 60:40) on hematological, inflammatory and some oxidative stress markers in high fat diet obese-induced Wistar rats. Results showed that oil quality indices were all within the norm and of good quality. The analysis of oxidative stress parameters revealed that the catalase activity was not significantly different amongst all test groups in kidney and heart. GSH activity amongst test groups of kidney homogenates was not significantly different. NO and MDA levels showed a significant difference for organ homogenates amongst all test groups. Hematological parameters of LYM, RBC, HGB, MCH, MCHC, MPV, PCT, and PDW had no significant difference amongst the test groups. Serum levels of TNF-α, INF- γ, IL-1β and IL-6 were significantly higher in the negative control group. These markers improved in the test groups treated with the oils. Therefore, it can be concluded that multisource edible oils from palm oil and African walnut oil have a role in the protection from obesity and its effects on oxidative stress, hematology, and inflammatory cytokines. It will be important in future studies to look into the effect of such formulation on the lipid profile and serum enzymes of rats.

Declarations

Ethical Statement

This is to inform you that in this study, animals were involved. Animals were cared for and used in agreement with international standard guidelines for animal use. In order to carry out this study, an ethical clearance for animal handling and care was obtained from the University of Buea - Institutional Animal Care and Use Committee (IACUC) with permit UB-IACUC No 02/2024.

Author Contribution Statement

Fabrice Tonfack Djikeng: Conceptualization, Data collection, investigation, Methodology, formal analysis, writing the original draft.

Mundi Eunice-Laura Lemnyuy: Data collection, investigation, Methodology, formal analysis, writing the original draft.

Veshe-Teh Zemoh Sylvia Ninying: Conceptualization, Data collection, investigation, Methodology, formal analysis, writing the original draft.

Tiencheu Bernard: Project administration, Conceptualization, Supervision, review and editing.

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Conflict of interest

The authors declare no conflict of interest

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