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Dietary Crude and Emulsified *Elaeis guineensis* Mesocarp Oil Improve Male Reproductive Health in Wistar Rats: A Phytotherapeutic Perspective

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ABSTRACT

Background: Male infertility is an increasing global health concern. In African traditional medicine, red palm oil is used alone or with herbs or honey to enhance male fertility by improving sperm quality and libido. Its rich bioactive composition supports its therapeutic potential, yet its oily consistency may limit absorption and bioavailability.

Aim of the Study: This study evaluated the effects of crude palm oil (EGCMO) and its emulsified form (EGEMO) on male reproductive parameters in Wistar rats to validate their ethnomedicinal relevance and improve pharmacological applicability.

Materials and Methods: The antioxidant capacities (1,1-diphenyl-2-picrylhydrazyl [DPPH], 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) [ABTS], and ferric reducing antioxidant power [FRAP]) and phenolic contents of EGCMO and EGEMO were determined in vitro. Male Wistar rats (10–12 weeks) received 1 or 2 mL/100 g body weight of EGCMO or EGEMO. Controls received distilled water, while a reference group received testosterone enanthate (4 mg/kg, intramuscular, every 3 days). Treatments lasted 30 or 60 days, after which reproductive organs and blood were analyzed for biochemical, hormonal, and histological parameters.

Results: EGCMO showed higher FRAP and EGEMO greater ABTS activity. EGCMO (2 mL/100 g) and both EGEMO doses significantly increased daily and total sperm production ($p < 0.05$) and improved sperm motility compared to controls and testosterone-treated rats. EGEMO enhanced catalase (CAT) activity after 30 days, while EGCMO did so after 60 days, with no adverse lipid effects.

Conclusions: Both EGCMO and EGEMO improved male reproductive function, supporting the ethnomedicinal use of palm oil against infertility. The emulsified form enhanced efficacy and bioavailability, bridging traditional African remedies with modern nutraceutical innovation.

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1 | Introduction

Infertility is defined as the inability of a couple to achieve clinical pregnancy after 12 months of regular, unprotected sexual intercourse [1]. According to the World Health Organization [2], ~17.5% of the global adult population, equivalent to one in six individuals, experience infertility at some point in their lives. Globally, infertility affects ~15% of couples, equating to around 186 million individuals [3]. In Africa, the prevalence of infertility is notably high, with rates ranging from 15% to 30% in various regions [4]. For instance, studies have reported rates ranging from 9% in Gambia to between 20% and 30% in Nigeria [5]. In Cameroon, a study conducted at the Central Hospital of Yaoundé found a hospital-based prevalence of male infertility at 10.08% [6]. These figures underscore the substantial impact of infertility on reproductive health both globally and within the African context. Male factors are solely responsible for about 20% of infertility cases and contribute to an additional 30%–40% of cases, making male infertility a significant contributor to reproductive challenges [3]. Women are frequently blamed for reproductive failure [7]. Many men avoid fertility testing due to stigma and ego concerns, and few voluntarily seek diagnosis. This reluctance is often rooted in societal perceptions that equate male fertility with masculinity, leading to feelings of shame and inadequacy among affected individuals. Consequently, men may delay or avoid seeking medical evaluation and treatment, further exacerbating the issue [8].

Male infertility is often associated with hormonal imbalances and oxidative stress, an imbalance between the overproduction of reactive oxygen species (ROS) and the body's antioxidant defenses. Notably, between 30% and 80% of male infertility patients produce excessive ROS in their ejaculate, underscoring the critical role of oxidative stress in male reproductive health [9, 10]. Factors such as obesity, smoking, excessive alcohol consumption, and exposure to ionizing radiation contribute to ROS generation. Oxidative stress can damage cellular components, including proteins, lipids, and DNA, compromising sperm quality and reproductive success [11].

Natural substances, especially those with no known toxicity, offer discreet alternatives that may improve male fertility without requiring formal diagnosis. Palm oil, extracted from the pulp of *Elaeis guineensis*, is rich in beneficial fatty acids and bioactive compounds. It contains 40%–42% palmitic acid, 8%–10% polyunsaturated fatty acids (PUFAs) (mainly linoleic acid), and 3%–5% stearic acid. In West and Central Africa, including Cameroon and Nigeria, palm oil and its derivatives are widely used in traditional remedies to support male fertility. In Nigeria, traditional healers administer palm oil either neat or blended with aphrodisiac seeds such as *Garcinia kola* or *Aframomum melegueta*, often sweetened with honey, aiming to enhance libido and sperm quality [12, 13]. Ethnobotanical surveys in Cameroon report that, while medicinal plants constitute the primary therapeutic agents, palm oil frequently serves as an important solvent or carrier in herbal fertility preparations, facilitating the extraction and bioavailability of active compounds [14]. Additionally, palm oil is combined with palm wine or honey in fertility tonics administered orally or via enema in some Cameroonian communities [15]. Palm mesocarp oil from *E. guineensis* is also traditionally attributed with aphrodisiac properties and used to treat male sexual dysfunction and infertility [15]. The potential benefits of palm oil may relate to its rich content of carotenoids, tocotrienols, and sterols, which enhance the stability and absorption of bioactive phytochemicals, supporting reproductive health.

While modern science has not fully confirmed these uses, early research suggests that palm oil's antioxidants and anti-inflammatory effects could help explain its traditional role in fighting infertility [16] among many diseases reported in ethnobotanical researches [15, 17]. Palm oil supports oxidative balance, protects testicular cells, and enhances both steroidogenesis and spermatogenesis. Notably, it is the richest natural plant source of vitamin E (70%–80% tocotrienols) and is recognized for its antioxidant potential [18, 19]. It is also a rich source of carotenoids, particularly beta-carotene, contributing to vitamin A synthesis, an essential factor for sex steroid production, spermatogenesis, and embryogenesis [20, 21]. Red palm oil has demonstrated protective effects against heavy metal-induced testicular damage and has been shown to improve glutathione levels and sperm parameters [22]. Furthermore, Aboua et al. [23] found that 60-day oral supplementation prevents peroxide-induced sperm apoptosis by inhibiting caspases 3 and 7 and reducing p53 activation. Although several studies report that crude and processed palm oil products contain phenolic compounds with measurable in vitro antioxidant activity (such as 1,1-diphenyl-2-picrylhydrazyl [DPPH], 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) [ABTS], or ferric reducing antioxidant power [FRAP]) such assays represent only preliminary evidence and may not reflect biological efficacy in vivo. Complex factors including digestion, metabolic conversion, tissue uptake, and interaction with endogenous antioxidant systems can substantially modify the actual physiological impact of these constituents. Therefore, in vitro antioxidant findings on palm oil extracts must be confirmed by in vivo models (by measuring biomarkers such as malondialdehyde [MDA], superoxide dismutase [SOD], catalase [CAT], or GSH) to determine whether the observed radical-scavenging potential truly contributes to protecting reproductive or systemic tissues from oxidative stress. This need for in vivo verification has been emphasized broadly in antioxidant research, as in vitro tests alone may overestimate activity and fail to predict biological relevance [24–26]. Polyphenols and flavonoids are other key bioactive constituents of crude palm oil, and their quantification is essential when assessing its potential benefits on male fertility. These compounds exert strong antioxidant effects that help neutralize ROS, a major contributor to sperm dysfunction and impaired steroidogenesis. Several studies have demonstrated that dietary polyphenols improve testicular antioxidant status, enhance Leydig cell function, and protect sperm membrane integrity, mechanisms directly relevant to fertility enhancement [21, 26]. Moreover, palm oil-derived phenolics such as catechins and p-coumaric acid have been shown to attenuate oxidative stress and improve reproductive outcomes in experimental models, reinforcing the need to assay their levels when evaluating palm oil as a fertility-promoting natural product [25].

Given the high cost and side effects of conventional therapies, nutraceuticals such as red palm oil are increasingly explored for treating male infertility. Although the efficacy and safety of crude palm oil (EGCMO) in enhancing male reproductive function have been previously documented [27], its direct consumption is often limited by its dense oily texture, difficult palatability, and reduced gastrointestinal dispersion. These characteristics may hinder the release and absorption of key bioactive constituents, thereby constraining its therapeutic potential. Emulsification offers a practical strategy to overcome these limitations by improving digestibility, enhancing sensory acceptability, and increasing the bioavailability of lipophilic antioxidants and phenolic compounds. Moreover, emulsified formulations are known to facilitate better micellar

incorporation and intestinal uptake, suggesting that an emulsified palm oil matrix could potentiate the fertility-enhancing effects traditionally attributed to EGCMO. Despite these promising theoretical advantages, no study to date has systematically compared the reproductive and metabolic effects of EGCMO and its emulsified form (EGEMO). The present work therefore aimed to clarify whether emulsification provides measurable benefits over crude oil in promoting male fertility, improving antioxidant defense, and maintaining metabolic safety. It was designed to evaluate and compare the effects of crude palm oil and its emulsion on fertility parameters in Wistar male rats. Additionally, their in vitro antioxidant capacities were assessed to explore potential mechanisms of action.

2 | Materials and Methods

2.1 | Reagents

- DPPH ≥96% pure,
- Folin–Ciocalteu reagent,
- ferrous chloride,
- ABTS 98% pure,
- potassium ferricyanide ($K_3Fe(CN)_6$) 99.5% pure,
- sodium carbonate (Na_2CO_3),
- potassium persulfate,
- ascorbic acid 99.5% pure.

These reagents, of analytical grade, were purchased from Massande Chemistry Lab, China. All other chemicals and solvents used were of analytical grade, unless otherwise specified.

2.2 | Plant Material

Crude palm oil (EGCMO) was freshly obtained in December 2023 from a production facility of SOCAPALM (*Société Camerounaise des Palmeraies*), located in Tillo, Douala (P.O. Box 691, 4.17044° N; 9.55767° E). The oil was collected in a sterile 1 L flask and transported aseptically to the laboratory. Upon arrival, it was aliquoted and stored at 4°C. Fresh samples of crude palm oil (EGCMO) and its emulsion (EGEMO) were subsequently prepared every 2 days throughout the study from these aliquots for administration to the experimental animals.

2.3 | Production of Emulsified Palm Oil (EGEMO)

EGEMO was prepared at room temperature using an oil-to-water ratio of 7:3 and 4% Tween 80 as an emulsifier. The mixture was homogenized at 250 × *g* until a uniform emulsion was obtained (the homogenization lasted approximatively 20 min). The resulting emulsion was transferred into sterile bottles and pasteurized at 70°C for 10 min in a water bath following the method described by Wu et al. [28].

2.4 | Experimental Animals

The experiment was conducted using 65 albino Wistar rats, aged 10–12 weeks and weighing between 150 and 210 g. The animals were bred in the animal facility of the Department of Biochemistry, University of Douala, and maintained under controlled environmental conditions at room temperature (26 ± 2°C), with adequate ventilation and a natural light–dark cycle of ~12 h each. The rats were provided with a standard laboratory diet and had access to tap water ad libitum.

2.5 | Evaluation of the Fatty Acid Profile of EGCMO and EGEMO by GC-FID Method

The fatty acid composition of EGCMO and EGEMO was determined after lipid extraction and transesterification into fatty acid methyl esters (FAMEs). Total lipids were extracted using a modified Folch method with chloroform: methanol (2:1, v/v), followed by phase separation with 0.9% saline and drying over anhydrous sodium sulfate. The lipid extract was transesterified using methanolic potassium hydroxide and boron trifluoride in methanol. FAMEs were extracted with n-hexane, evaporated, and 1 µL was injected into a GC-FID system equipped with a BPX70 capillary column (30 m × 0.25 mm × 0.25 µm). Helium was used as a carrier gas at 1.25 mL·min^{-1}. The oven temperature ranged from 140 to 240°C according to manufacturer specifications. Fatty acids were identified by comparison with standard FAME retention times and expressed as relative percentages. Supelco 37-Component FAME Mix (Sigma–Aldrich) was used as reference.

2.6 | Evaluation of Antiradical Power and Antioxidant Activity

2.6.1 | Radical-Scavenging Activity

2.6.1.1 | DPPH Assay. DPPH radical-scavenging activity of EGCMO and EGEMO was determined using the method of Brand-Williams et al. [29] with slight modifications. Briefly, 1 mL of sample was mixed with 4 mL of methanolic DPPH solution (0.005 mg/mL; methanol/water 80:20, v/v), vortexed, and incubated in the dark for 30 min. Absorbance was measured at 517 nm using an AQX1MAN-FR Orion AquaMate 7100/8100 spectrophotometer. Vitamin C was used as reference.

$$\text{Scavenging activity } (\%) = \left[\left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right)/\text{Abs}_{\text{control}}\right] \times 100. \quad (1)$$

SC_{50} values were determined from inhibition curves. All assays were performed in triplicate ($n = 3$).

2.6.1.2 | ABTS Assay. ABTS radical-scavenging activity was evaluated according to Re et al. [30]. The $ABTS^{\cdot+}$ solution was prepared by mixing 7 mM ABTS with 2.4 mM potassium persulfate and incubating for 14 h in the dark. Before use, it was diluted to an absorbance of 0.70 ± 0.02 at 734 nm. Then, 0.5 mL of sample was mixed with 2 mL of ABTS solution (methanol/water 80:20, v/v) and incubated for 7 min at room temperature in the dark. Absorbance was measured at 734 nm. Ascorbic acid served as reference.

$$\%\ \text{Inhibition} = [(A_0 - A_1)/A_0] \times 100. \quad (2)$$

SC_{50} values were calculated from inhibition curves. All measurements were performed in triplicate ($n = 3$).

2.6.2 | FRAP

FRAP was assessed following Nadri et al. [31] with slight modifications. Briefly, 1 mL of sample was mixed with 4 mL methanol/water (80:20, v/v) and centrifuged (4000 × *g*, 10 min). An aliquot (0.4 mL) of the supernatant was mixed with 1 mL phosphate buffer (0.2 M, pH 6.6) and 1 mL potassium ferricyanide (1%), then incubated at 50°C for 30 min. After addition of 1 mL trichloroacetic acid (10%) and centrifugation (3000 × *g*, 10 min), the supernatant was collected and mixed with 0.2 mL ferric chloride (0.1%). After 30 min incubation in the dark, absorbance was measured at 700 nm.

Antioxidant activity was expressed as IC_{50} (absorbance = 0.5), determined from the regression curve using ascorbic acid as a standard. All assays were conducted in triplicate ($n = 3$).

2.7 | Evaluation of Phenolic Compounds in EGCMO and EGEMO

2.7.1 | Total Phenolic Content (TPC)

TPC was determined using the Folin–Ciocalteu method described by Chew et al. [32]. Briefly, 1 mL of sample was mixed with 4 mL methanol/water (80:20, v/v) and centrifuged (4000 × *g*, 10 min). 1 mL of the supernatant was combined with 5 mL Folin–Ciocalteu reagent (1:10) and 4 mL sodium carbonate (75 g/L). After vortexing and incubation in the dark for 2 h at room temperature, absorbance was measured at 765 nm using an AQX1MAN-FR Orion AquaMate 7100/8100 spectrophotometer. Results were expressed as mg ascorbic acid equivalents per mL of oil (mg AAE/mL), based on an ascorbic acid calibration curve (0–2 mg/mL).

2.7.2 | Total Flavonoid Content (TFC)

TFC was determined using the aluminum chloride colorimetric method of Srivastava et al. [33]. Briefly, 0.5 mL of 2% $AlCl_3$ (ethanol) was added to 0.5 mL of sample supernatant, followed by 0.1 mL sodium acetate and 2.8 mL distilled water. After 1 h incubation at room temperature, absorbance was measured at 750 nm. Results were expressed as mg quercetin equivalents per mL of oil (mg QE/mL), using a quercetin calibration curve.

2.8 | Experimental Protocol

A total of 65 animals were randomly assigned to seven groups (Figure 1). The day 0 group ($n = 5$) received no treatment and was sacrificed at baseline. The control group ($n = 10$) received distilled water (1 mL/100 g body weight). Two groups received crude palm oil (EGCMO1 and EGCMO2; 1 mL/100 g and 2 mL/100 g, respectively), while two others received emulsified palm oil (EGEMO1 and EGEMO2) at the same doses ($n = 10$ each). The reference group received intramuscular testosterone enanthate (4 mg/kg every 3 days).

Treatments were administered for 30 and 60 days. At each time point, five animals per group were sacrificed by intraperitoneal injection of a ketamine–diazepam mixture (80 mg/kg and

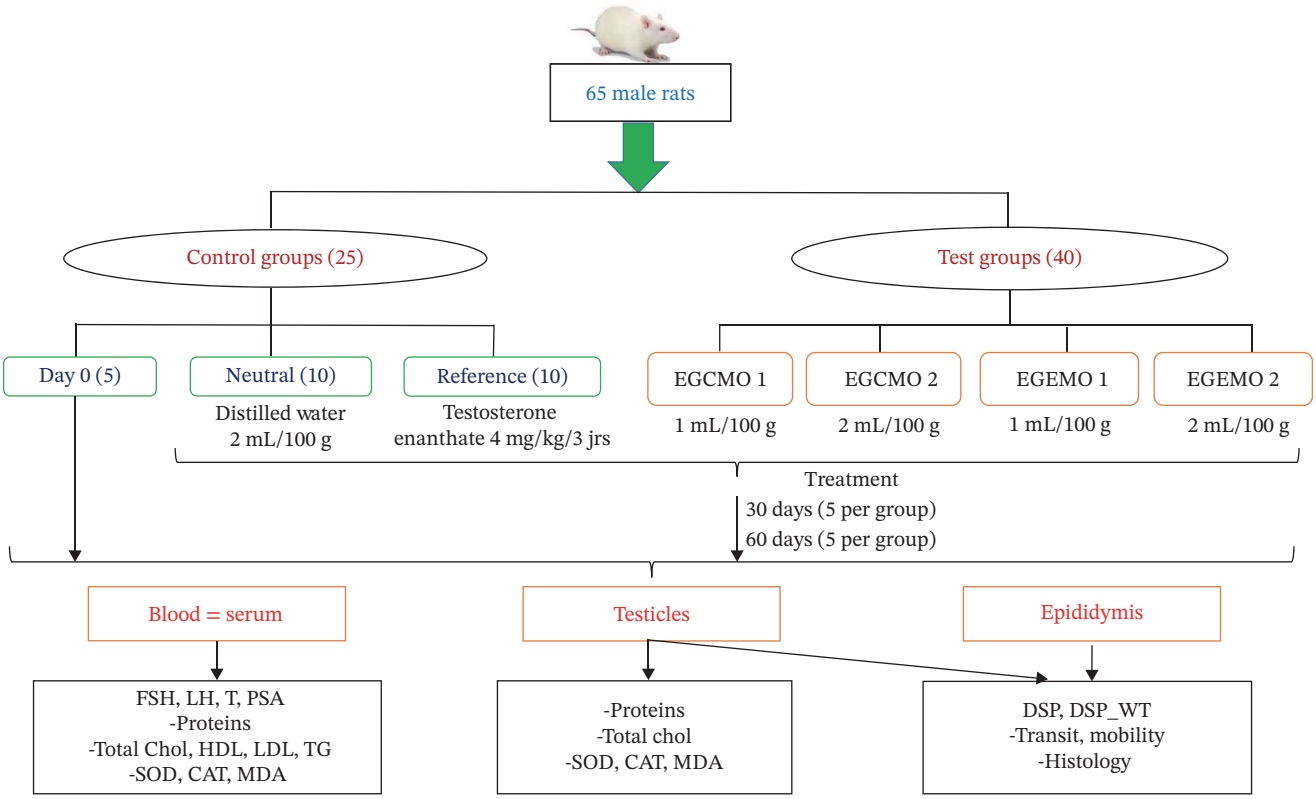


FIGURE 1 | Schematic representation of the different treatments on rats and analysis. The number in brackets represent the number of animals considered per experimental group. CAT, catalase; Chol, cholesterol; DSP, daily sperm production; DSP_WT, effective sperm production; EGCMO, crude *Elaeis guineensis* mesocarp oil; EGEMO, emulsified *Elaeis guineensis* mesocarp oil; FSH, follicle-stimulating hormone; HDL, high density lipoprotein; LDL, low density lipoprotein; LH, luteinizing hormone; MDA, malondialdehyde; PSA, prostate-specific antigen; SOD, superoxide dismutase; T, testosterone; TG, triglycerides.

5 mg/kg, respectively). Blood was collected by cardiac puncture for serum preparation, and reproductive organs were excised, weighed, and processed for biochemical, physiological, and histological analyses (Figure 1).

2.9 | Preparation of Organ Homogenates

2.9.1 | Testicular Homogenates

The right testis was dissected, decapsulated, divided into two parts, and weighed. One portion was homogenized (15%, w/v) in 50 mM potassium phosphate buffer (pH 7.4), centrifuged (3000 × *g*, 15 min), and the supernatant collected, aliquoted, and stored at −20°C for up to 2 weeks for the analysis of testosterone, proteins, cholesterol, and oxidative stress markers.

2.9.2 | Epididymal Homogenates

The right epididymal head was processed using the same procedure as described for the testis for subsequent biochemical analyses.

2.10 | Evaluation of Spermatic Parameters

Sperm count in the left testis was determined according to Lembè et al. [34]. After removal of the tunica albuginea, the tissue was homogenized (15%, w/v) in Triton X-100 using an EKO Maxx homogenizer (2 min). 1 mL of homogenate was diluted with 9 mL Triton X-100, and 10 µL was loaded onto a Neubauer Improved chamber for counting at 100 × magnification. Only sperm heads were counted across 25 large squares.

Daily sperm production (DSP) was calculated by dividing the total sperm count by the dilution factor (6.4). Effective sperm production (DSP_WT) was obtained by normalizing DSP to testicular weight, as described by Lembè et al. [34].

2.11 | Sperm Motility and Epididymal Transit

Sperm motility and transit were assessed following Ngoula et al. [35]. The left epididymal tail was excised and minced in 2 mL of 0.9% NaCl. A 10 µL aliquot was examined at 400 × magnification (Olympus CX23 microscope). Motile and immotile sperm were counted in five random fields, and motility was calculated:

$$\text{Motility}\ (\%) = (\text{motile sperm}/\text{total sperm}) \times 100. \quad (3)$$

Epididymal transit time was estimated by dividing total epididymal sperm count by DSP, as described by Lembè et al. [34].

2.12 | Effect on Serum Sex Hormone Levels

2.12.1 | Testosterone

Testosterone levels were determined by competitive ELISA following Fornes et al. [36]. Sample testosterone competed with HRP-conjugated testosterone for binding to precoated antibodies. After incubation and washing, substrate addition produced a color inversely proportional to testosterone concentration. Absorbance was measured at 450 nm using a BIUREX kit.

2.12.2 | Prostate-Specific Antigen (PSA)

PSA levels were measured using a sandwich ELISA (GBC kit). Samples were added to wells precoated with anti-PSA antibodies, followed by a biotinylated antibody and HRP-conjugated avidin. After incubation and washing, TMB substrate was added, producing a blue color proportional to PSA concentration, which turned yellow after acid addition. Absorbance was read at 450 nm, and concentrations were determined from a standard curve.

2.12.3 | Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH)

LH and FSH levels were quantified using sandwich ELISA (Elabscience kit). Samples were incubated in wells precoated with specific antibodies, followed by biotinylated detection antibodies and HRP-conjugated avidin. After washing, substrate addition produced a color proportional to hormone levels. The reaction was stopped, and absorbance was measured at 450 nm (LabSystems Multiskan RC). Concentrations were determined using standard curves.

2.13 | Measurement of Oxidative Stress Parameters

2.13.1 | Lipid Peroxidation (MDA Levels)

Lipid peroxidation was assessed by measuring MDA following Yoshioka et al. [37]. Briefly, 250 µL of serum or tissue homogenate was incubated at 95°C with 1.25 mL trichloroacetic acid (20%) and 500 µL thiobarbituric acid (0.67%). After cooling, 4 mL n-butanol was added, and the mixture was centrifuged (900 × *g*, 10 min). Absorbance of the organic phase was measured at 532 nm. MDA levels were expressed as nmol/mg protein using:

$$\text{MDA} = (\text{OD} \times d \times 1000)/(\varepsilon \times L \times X), \quad (4)$$

where OD is absorbance, d is the dilution factor, $\varepsilon = 1.56 \times 10^5$ $M^{-1}\,cm^{-1}$, L is the path length, and X is the protein concentration.

2.13.2 | SOD Activity

SOD activity was determined by the adrenaline autooxidation method, based on inhibition of adrenochrome formation in alkaline medium. Briefly, 134 µL of sample was mixed with 1666 µL carbonate buffer (0.05 M, pH 10.2), and the reaction was initiated with 200 µL adrenaline (0.3 mM). Absorbance at 480 nm was recorded at 20 and 80 s. One unit of SOD corresponds to 50% inhibition of adrenaline autooxidation per minute. Results were expressed as U/mg protein.

2.13.3 | CAT Activity

CAT activity was measured according to Clairborne [38] by monitoring H_2O_2 decomposition at 240 nm. The reaction mixture contained 80 µL sample, 780 µL phosphate buffer (100 mM, pH 7.8), and 200 µL H_2O_2 (75 mM). The decrease in absorbance was recorded for 90 s, and activity was calculated as follows:

$$\text{CAT} = (\Delta\text{OD} \times d)/(0.02 \times \varepsilon \times L \times X), \quad (5)$$

where $\varepsilon = 0.043\ mM^{-1}\,cm^{-1}$. Results were expressed as U/mg protein.

2.14 | Determination of Lipid Parameters and Serum Proteins

2.14.1 | Lipid Profile

Total cholesterol was measured using the CHOD-PAP enzymatic method coupled with the Trinder reaction [39]. HDL-C was determined after precipitation of LDL, VLDL, and chylomicrons using phosphotungstic acid and magnesium chloride, followed by centrifugation (3000 × *g*, 10 min), and cholesterol quantification in the supernatant.

Triglycerides were determined using an oxidase–peroxidase enzymatic method, with absorbance measured at 540 nm. LDL-C was calculated using the Friedewald formula [40].

2.14.2 | Serum Protein Quantification

Total protein was determined by the biuret method [41], based on the formation of a violet complex with Cu^{2+} in an alkaline medium. Absorbance was measured at 540 nm. All biochemical analyses were performed according to BIUREX kit protocols.

2.15 | Histological Examination

The remaining halves of the right testis and epididymis were fixed in Bouin's solution (<1 week), dehydrated in graded ethanol (70%–100%), cleared in xylene, and embedded in paraffin. Sections (7 μm) were obtained using a Reichert-Jung 2030 microtome, mounted on slides, and dried at 60°C for 30–40 min. After hematoxylin–eosin staining, sections were examined at 100 × magnification using a Leitz Wetzlar microscope. Images were captured with a Celestron 44421 camera and analyzed on a Toshiba Tecra A9 computer.

2.16 | Ethical Considerations

The study commenced with administrative procedures, including the drafting and approval of the research protocol by the Scientific Committee of the Faculty. Ethical clearance was subsequently obtained from the Institutional Ethics Committee of the University of Douala under Reference Number 4103 CEI-UDO/02/2024/M. The rules of the ARRIVE guidelines 2.0 of the National Centre for the Replacement, Refinement and Reduction of animals in research (NC3R[s]) were carefully respected through this research [42].

2.17 | Statistical Analysis

Data were recorded in Microsoft Excel and analyzed using SPSS software Version 26.0 (2019). Results are expressed as mean ± standard error of the mean (SEM). Plots and histograms were drawn with Microsoft Excel. Prior to analysis, the normality of data distribution and homogeneity of variances were assessed using the Shapiro–Wilk test and Levene's test, respectively. The effects of treatments on the measured parameters were then assessed using one-way analysis of variance (ANOVA). When significant differences were detected ($p < 0.05$), Fisher's least significant difference (LSD) post hoc test was applied to determine intergroup differences. Group-wise comparisons were made between the treatment groups and the corresponding control groups. A *p*-value less than 0.05 was considered statistically significant [43].

3 | Results

3.1 | Fatty Acid Profile of EGCMO and EGEMO

The fatty acid profiles (Table 1) showed that both EGCMO and EGEMO were rich in palmitic and oleic acids. PUFAs, including linoleic and linolenic acids, were present in both samples, with a predominance of omega-6. EGCMO exhibited a higher omega-6/omega-3 ratio (33.33) compared to EGEMO.

3.2 | Antioxidant Potential of EGCMO and EGEMO

The antioxidant activities of EGCMO and EGEMO, compared with vitamin C, are shown in Figure 2.

TABLE 1 | Quantitative profile of fatty acids in EGCMO and EGEMO.

Code	Name	Crude *Elaeis guineensis* mesocarp oil	Emulsified *Elaeis guineensis* mesocarp oil
		Percentages (%)	
C12:0	Lauric acid	0.2	0.19
C14:0	Myristic acid	1.2	1.15
C16:0	Palmitic acid	42	40.3
C16:1	Palmitoleic acid	0.3	0.32
C18:0	Stearic acid	5.1	4.8
C18:1 n-9	Oleic acid	39.9	45.5
C18:2 n-6	Linoleic acid	10	8.7
C18:3 n-3	Linolenic acid	0.3	0.29
C20:0	Arachidic acid	0.5	0.47
C20:1	Gadoleic acid	0.3	0.31

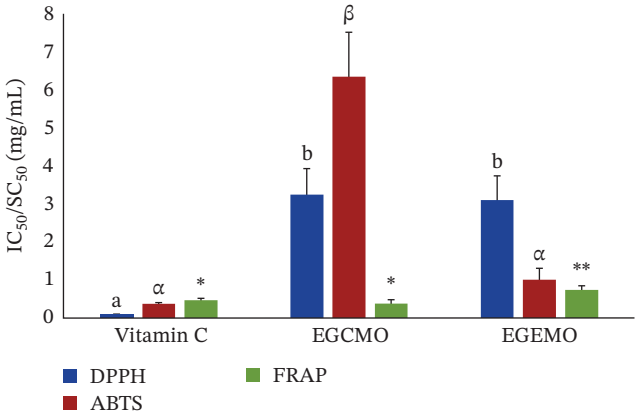


FIGURE 2 | Antioxidant activities at DPPH, ABTS, and FRAP of EGCMO and EGEMO. EGCMO, crude *Elaeis guineensis* mesocarp oil; EGEMO, emulsified *Elaeis guineensis* mesocarp oil. The histograms presented are the means ± SEM of three repetitions, The different letters, symbols or stars in superscript respectively present significant differences between DPPH, ABTS, and FRAP values of different tested substances at the threshold of $p < 0.05$ (Fisher LSD test).

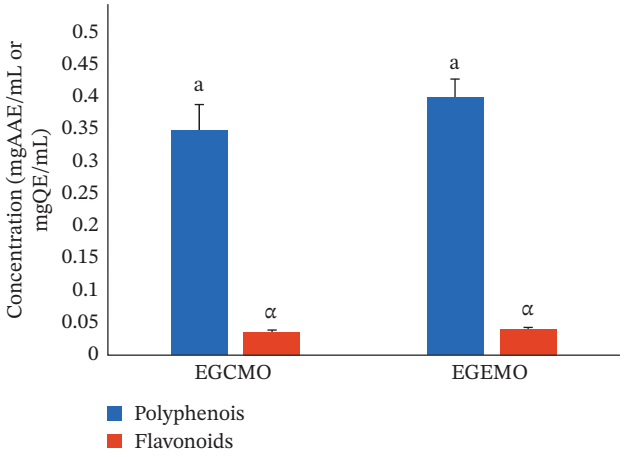


FIGURE 3 | Level of polyphenols and flavonoids in EGCMO and EGEMO. EGCMO, crude *Elaeis guineensis* mesocarp oil, EGEMO, emulsified *Elaeis guineensis* mesocarp oil. The histograms presented are the means ± SEM of three repetitions; same letters or symbols show variations not significant between the values represented as histograms (Fisher LSD test).

3.2.1 | DPPH Assay

Vitamin C showed the lowest SC_{50}, indicating the highest activity. EGCMO and EGEMO exhibited higher SC_{50} values, with no significant difference between them, suggesting similar DPPH scavenging capacity.

3.2.2 | ABTS Assay

EGEMO displayed a significantly lower SC_{50} than EGCMO, indicating stronger activity. EGCMO showed values comparable to vitamin C, suggesting substantial antioxidant potential via this pathway.

3.2.3 | FRAP Assay

EGCMO exhibited reducing power comparable to vitamin C and significantly higher than EGEMO, indicating greater electron-donating capacity.

3.3 | Phenolic Compounds Content

As shown in Figure 3, no significant differences were observed between EGCMO and EGEMO for total polyphenols (0.3487 ± 0.0414 vs. 0.4013 ± 0.0277 mg AAE/mL) or flavonoids (0.0365 ± 0.0027 vs. 0.0403 ± 0.0017 mg QE/mL). This indicates that emulsification does not significantly affect these bioactive compounds, preserving the antioxidant-related properties of the oil.

3.4 | Effect of EGCMO and EGEMO on Animals Relative Masses

Body mass evolution is presented in Figure 4. All treated groups, except EGEMO2, showed an initial weight decrease up to day 20, followed by a progressive increase. A moderate rise was observed between days 33 and 47, and a significant increase from days 51 to 61. Testosterone-treated rats exhibited the highest weight gain compared to all other groups.

3.5 | Effect of EGCMO and EGEMO on the Relative Mass of Some Reproductive Organs

Table 2 summarizes organ weight changes after 30 and 60 days.

Testicular mass significantly decreased in the control group compared to day 0, whereas EGCMO and EGEMO treatments induced significant increases at both time points. In contrast, testosterone administration markedly reduced testicular mass. No differences were observed among oil-treated groups.

Epididymal mass remained unchanged in controls but increased significantly in oil-treated groups compared to controls and testosterone at day 30. At day 60, a significant increase persisted only in the EGEMO1 group.

For seminal vesicles, no change was observed in controls at day 30, but an increase occurred at day 60. Oil-treated groups showed increased mass at day 30 but a significant decrease at day 60 compared to both control and testosterone groups, with no differences between treatments.

3.6 | Effects on Testicular Sperm Parameters

Table 3 summarizes DSP and effective sperm production after 30 and 60 days.

DSP significantly decreased in the testosterone-treated group compared to controls at both time points. In contrast, EGCMO2, EGEMO1, and EGEMO2 significantly increased DSP relative to the neutral control. Notably, EGEMO1 showed higher DSP than EGCMO1 at day 30, suggesting a formulation-related effect.

Similarly, effective sperm production decreased in the testosterone group but increased significantly in the EGCMO2, EGEMO1, and EGEMO2 groups compared to both baseline (day 0) and controls. At day 30, EGEMO1 exhibited a significantly greater effect than EGCMO1, confirming its superior efficacy at this dose.

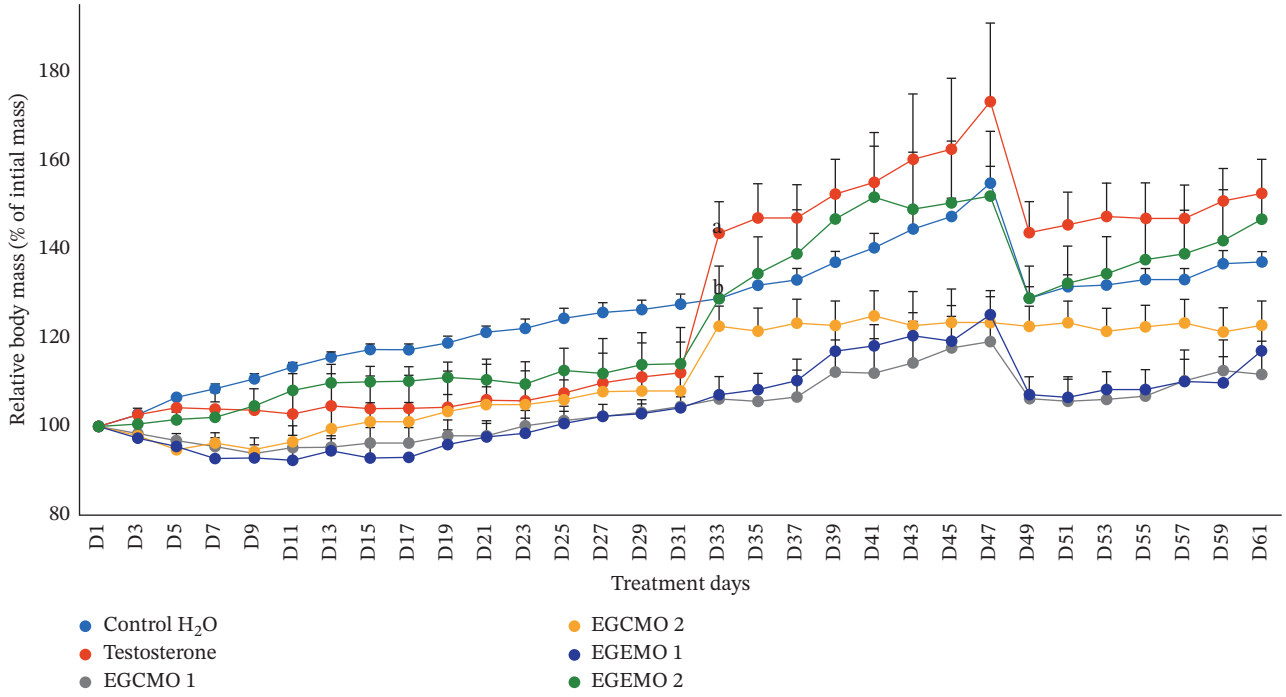


FIGURE 4 | Evolution of the body mass in experimental animals. Each dot on the curves represent the means ± SEM of the values of five animals. The different superscript letters in the columns, a, b, c,… represent significant differences between the values of the various experimental groups at the threshold of $p < 0.05$ (Fisher LSD test).

TABLE 2 | Effect of *Elaeis guineensis* mesocarp oil and its emulsion on the relative masses of some reproductive organs.

Treatment duration	Experimental groups	Testis (mg/g)	Epididymis (mg/g)	Seminal vesicles (mg/g)	Prostate (mg/g)
—	Day 0	0.56 ± 0.06^{ad}	0.19 ± 0.02^{ac}	0.18 ± 0.05^{a}	0.08 ± 0.02^{a}
30 days	Control (H_2O)	0.42 ± 0.03^{b}	0.17 ± 0.01^{a}	0.17 ± 0.03^{a}	0.09 ± 0.02^{a}
	Testosterone (4 mg/kg/3 days)	0.15 ± 0.05^{c}	0.15 ± 0.07^{a}	0.35 ± 0.21^{b}	0.16 ± 0.06^{b}
	EGCMO 1 (1 mL/100 g)	0.59 ± 0.05^{ad}	0.39 ± 0.09^{bcd}	0.33 ± 0.11^{ab}	0.08 ± 0.03^{a}
	EGCMO 2 (2 mL/100 g)	0.64 ± 0.02^{a}	0.35 ± 0.08^{bc}	0.24 ± 0.03^{ab}	0.09 ± 0.02^{a}
	EGEMO 1 (1 mL/100 g)	0.62 ± 0.07^{ad}	0.46 ± 013^{bd}	0.29 ± 0.08^{ab}	0.12 ± 0.02^{b}
	EGEMO 2 (2 mL/100 g)	0.55 ± 0.17^{a}	0.30 ± 0.09^{c}	0.29 ± 0.16^{c}	0.14 ± 0.03^{b}
60 days	Control (H_2O)	0.49 ± 0.08^{bd}	0.24 ± 0.03^{ac}	0.60 ± 0.19^{c}	0.20 ± 0.04^{c}
	Testosterone (4 mg/kg/3 days)	0.18 ± 0.026^{c}	0.27 ± 0.12^{a}	0.61 ± 0.13^{ab}	0.16 ± 0.05^{bc}
	EGCMO 1 (1 mL/100 g)	0.53 ± 0.101^{d}	0.28 ± 0.05^{c}	0.23 ± 0.08^{ab}	0.10 ± 0.02^{a}
	EGCMO 2 (2 mL/100 g)	0.56 ± 0.04^{ad}	0.22 ± 0.026^{a}	0.21 ± 0.05^{ab}	0.19 ± 0.03^{bc}
	EGEMO 1 (1 mL/100 g)	0.50 ± 0.118^{b}	0.47 ± 0.19^{d}	0.24 ± 0.03^{ab}	0.11 ± 0.02^{a}
	EGEMO 2 (2 mL/100 g)	0.56 ± 0.06^{ad}	0.22 ± 0.03^{a}	0.25 ± 0.03^{ab}	0.10 ± 0.03^{a}

Note: The data represented are the means ± SEM of the values of five animals, each value is expressed in mg/g of animal's body weight on the last day of treatment. The different superscript letters in the columns, a, b, c,… represent significant differences between the values of the various experimental groups at the threshold of $p < 0.05$ (Fisher LSD test).
Abbreviations: EGCMO, crude *Elaeis guineensis* mesocarp oil; EGEMO, emulsified *Elaeis guineensis* mesocarp oil.

3.7 | Effects on Epididymal Sperm Parameters

Table 4 presents sperm motility and epididymal transit data.

Sperm motility significantly decreased in the testosterone group at both time points. Conversely, EGCMO1, EGCMO2, and EGEMO2 significantly improved motility compared to controls and testosterone-treated animals. At day 60, EGEMO1 showed markedly higher motility (92.00 ± 13.30) than EGCMO1 (52.60 ± 17.47), indicating a stronger effect of the emulsion. An increase in motility was also observed in the control group at day 60.

Epididymal transit significantly increased in the testosterone group, whereas both EGCMO and EGEMO reduced transit time compared

TABLE 3 | Daily production (DSP) and effective production (DSP_WT) of spermatozoa after administration of EGCMO and EGEMO in rats.

Treatment duration	Experimental groups	DSP ($\times 10^6$/g of testis)	DSP_WT ($\times 10^6$/g of testis)
—	Day 0	5.24 ± 0.27^{a}	5.93 ± 0.49^{afg}
30 days	Control (H_2O)	1.24 ± 0.08^{bc}	1.71 ± 0.10^{b}
	Testosterone (4 mg/kg/3 days)	0.19 ± 0.03^{b}	1.10 ± 0.41^{b}
	EGCMO 1 (1 mL/100 g)	2.36 ± 0.16^{ce}	3.60 ± 0.36^{cf}
	EGCMO 2 (2 mL/100 g)	6.29 ± 0.73^{ad}	7.07 ± 0.64^{ad}
	EGEMO 1 (1 mL/100 g)	7.50 ± 1.16^{d}	9.46 ± 0.50^{d}
	EGEMO 2 (2 mL/100 g)	5.67 ± 0.79^{a}	8.11 ± 0.62^{d}
60 days	Control (H_2O)	3.08 ± 0.79^{e}	2.83 ± 0.73^{c}
	Testosterone (4 mg/kg/3 days)	0.16 ± 0.00^{b}	0.41 ± 0.04^{e}
	EGCMO 1 (1 mL/100 g)	4.85 ± 0.69^{a}	5.09 ± 0.65^{fg}
	EGCMO 2 (2 mL/100 g)	4.85 ± 0.30^{a}	4.62 ± 0.09^{fg}
	EGEMO 1 (1 mL/100 g)	6.00 ± 0.41^{ad}	5.60 ± 0.22^{g}
	EGEMO 2 (2 mL/100 g)	5.73 ± 0.89^{a}	5.94 ± 0.58^{ag}

Note: The data represented are the means ± SEM of the values of five animals. The different letters in superscript, a, b, c,… represent significant differences between the values of the various experimental groups at the threshold of $p < 0.05$ (Fisher LSD test).
Abbreviations: EGCMO, crude *Elaeis guineensis* mesocarp oil; EGEMO, emulsified *Elaeis guineensis* mesocarp oil.

TABLE 4 | Percentage of motility and transit of spermatozoa after treatment with EGCMO and EGEMO oil in rats.

Treatment duration	Experimental group	Mobility (%)	Transit ($\times 10^6$/g of testis)
—	Day 0	22.80 ± 10.23^{ac}	4.05 ± 0.37^{a}
30 days	Control (H_2O)	5.00 ± 2.00^{b}	3.72 ± 0.77^{a}
	Testosterone (4 mg/kg/3 days)	0.00 ± 0.00^{b}	6.40 ± 0.23^{b}
	EGCMO 1 (1 mL/100 g)	13.40 ± 3.97^{ab}	2.02 ± 0.18^{cd}
	EGCMO 2 (2 mL/100 g)	27.60 ± 12.66^{c}	0.62 ± 0.10^{cd}
	EGEMO 1 (1 mL/100 g)	23.00 ± 09.74^{ac}	0.92 ± 0.29^{cd}
	EGEMO 2 (2 mL/100 g)	23.00 ± 9.74^{ac}	1.80 ± 0.65^{cd}
60 days	Control (H_2O)	41.60 ± 14.65^{d}	2.60 ± 0.67^{ad}
	Testosterone (4 mg/kg/3 days)	0.00 ± 0.00^{b}	5.61 ± 1.00^{b}
	EGCMO 1 (1 mL/100 g)	52.60 ± 17.47^{d}	3.27 ± 0.33^{ad}
	EGCMO 2 (2 mL/100 g)	90.00 ± 15.41^{e}	2.87 ± 0.24^{ad}
	EGEMO 1 (1 mL/100 g)	92.00 ± 13.03^{e}	1.99 ± 0.23^{d}
	EGEMO 2 (2 mL/100 g)	94.00 ± 8.94^{e}	3.51 ± 0.55^{a}

Note: The data presented are the means ± SEM of the values of five animals. The different letters in superscript, a, b, c,… represent significant differences between the values of the various experimental groups at the threshold of $p < 0.05$ (Fisher LSD).
Abbreviations: EGCMO, crude *Elaeis guineensis* mesocarp oil; EGEMO, emulsified *Elaeis guineensis* mesocarp oil.

to controls. EGEMO1 showed a lower transit rate than EGCMO at day 60, suggesting improved sperm maturation or retention.

3.8 | Histological Effects on Testis and Epididymis (30 Days)

Figure 5 shows epididymal histology after 30 days. All treated groups exhibited normal architecture comparable to controls, with intact epithelium and lumens containing abundant spermatozoa, indicating no histopathological alterations.

Figure 6 illustrates testicular histology. Control animals showed normal seminiferous tubule organization with all stages of spermatogenesis. EGCMO and EGEMO treatments did not induce structural alterations. However, EGEMO-treated rats displayed a dose-dependent increase in luminal sperm density, suggesting enhanced spermatogenic activity.

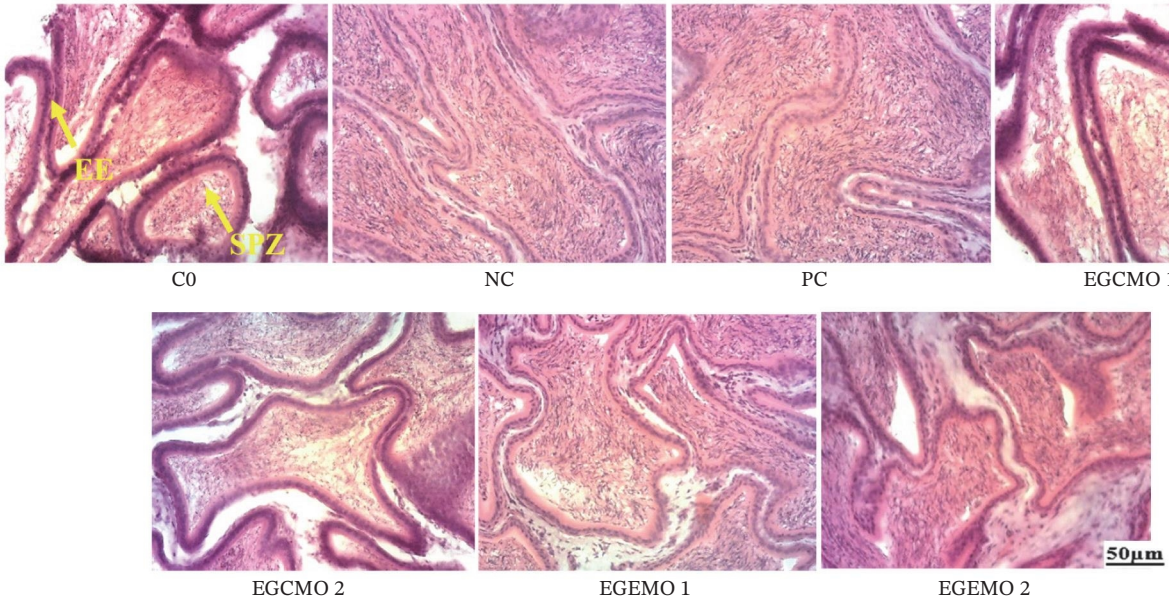


FIGURE 5 | Effects of 30-administration days of EGCMO and EGEMO on the epididymis structure. Hematoxylin–eosin staining; 100x magnification. C0, control day 0; EE, epididymal epithelium; EGCMO, crude *Elaeis guineensis* mesocarp oil; EGEMO, emulsified *Elaeis guineensis* mesocarp oil; NC, neutral control; PC, positive control (testosterone); Spz, seminiferous sperm cells.

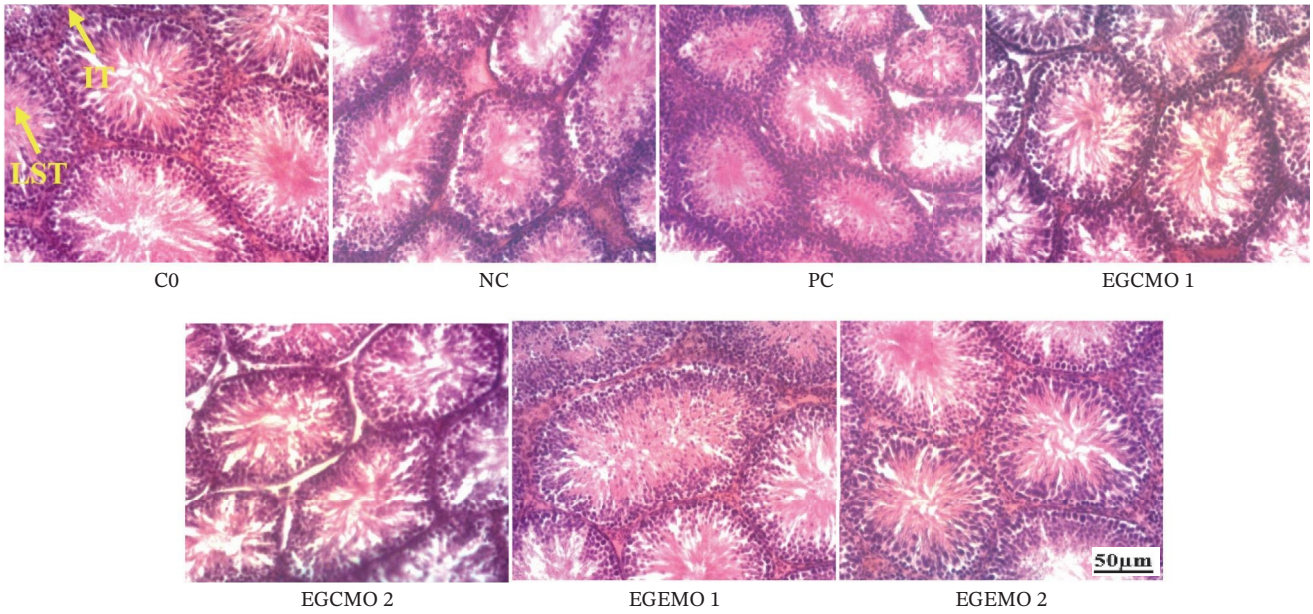


FIGURE 6 | Effects of EGCMO and EGEMO on the structure of the testicle after 30 days of administration. Hematoxylin–eosin staining; 100x magnification. CO, control day 0; EGCMO, crude *Elaeis guineensis* mesocarp oil; EGEMO, emulsified *Elaeis guineensis* mesocarp oil; IT, interstitial tissue; LST, lumen of the seminiferous tubule; NC, neutral control; PC, positive control (testosterone).

3.9 | Histological Effects After 60 Days

Figures 7 and 8 show epididymal and testicular histology after 60 days of treatment.

No histopathological alterations were observed in any treated group. The epididymis displayed normal architecture with intact epithelium and lumens densely filled with spermatozoa, comparable to controls. Similarly, seminiferous tubules retained normal organization across all groups. However, EGEMO-treated rats exhibited a dose-dependent increase in luminal sperm density, suggesting enhanced spermatogenic activity.

3.10 | Effects on Reproductive Hormonal Parameters

Table 5 summarizes serum testosterone, FSH, LH, and PSA levels.

After 30 days, testosterone levels increased significantly in testosterone, EGCMO2, EGEMO1, and EGEMO2 groups, while EGCMO1 showed lower levels compared to the reference group. At 60 days, testosterone increased in controls but decreased in EGCMO-treated groups, with all treatments remaining lower than the testosterone group.

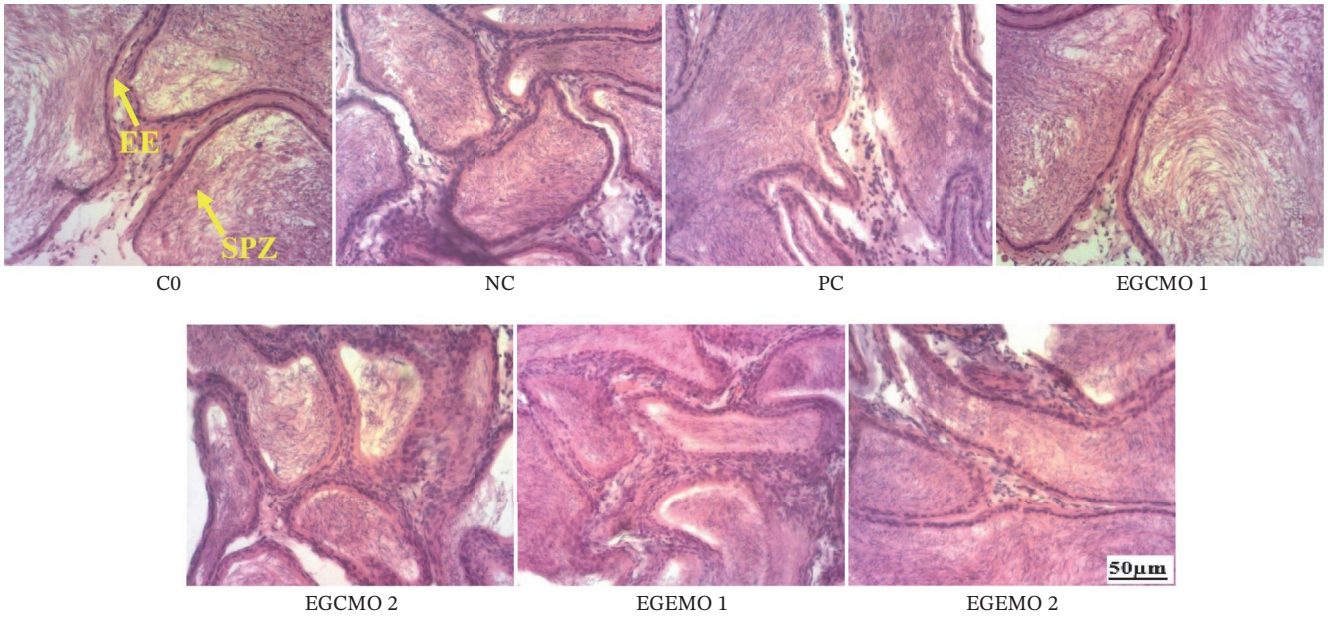


FIGURE 7 | Effects of 60-administration days of EGCMO and EGEMO on the epididymis structure. Hematoxylin–eosin staining; 100x magnification. CO, control day 0; EE, epididymal epithelium; EGCMO, crude *Elaeis guineensis* mesocarp oil; EGEMO, emulsified *Elaeis guineensis* mesocarp oil; NC, neutral control; PC, positive control (testosterone); Spz, seminiferous sperm cells.

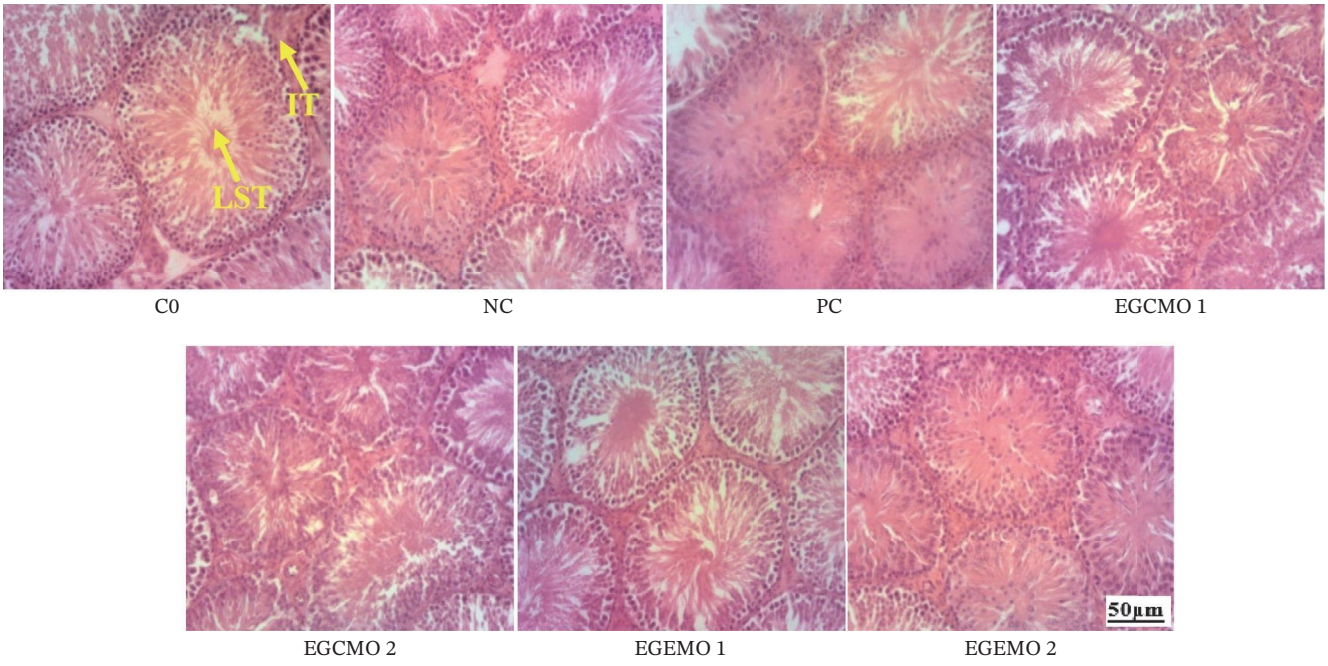


FIGURE 8 | Effects of EGCMO and EGEMO on the structure of the testicle after 60 days of administration. Hematoxylin–eosin staining; 100x magnification. CO, control day 0; EGCMO, crude *Elaeis guineensis* mesocarp oil; EGEMO, emulsified *Elaeis guineensis* mesocarp oil; IT, interstitial tissue; LTS, lumen of the seminiferous tubule; NC, neutral control; PC, positive control (testosterone).

FSH and LH levels remained stable in controls but significantly decreased in all EGCMO- and EGEMO-treated groups at both time points. In contrast, LH increased in the testosterone group.

PSA levels were unchanged at 30 days but increased in controls at 60 days. EGCMO and EGEMO significantly reduced PSA at both durations. EGEMO induced higher LH and PSA levels than EGCMO, indicating formulation-dependent effects.

3.11 | Effects on Serum Lipid Profile

As shown in Table 6, both EGCMO and EGEMO increased triglyceride levels compared to controls, with EGEMO1 showing a stronger effect at 30 days. Testosterone reduced triglycerides during the same period.

Total cholesterol increased with EGEMO1 at 30 days, while testosterone elevated cholesterol at 60 days. EGCMO1 appeared to better moderate this increase than EGCMO2.

TABLE 5 | Serum levels of male reproductive hormones in treated rats.

Treatment duration	Experimental groups	Testosterone (ng/mL)	LH (mlU/mL)	FSH (mlU/mL)	PSA (ng/mL)
—	Day 0	1.33 ± 0.06^{a}	1.41 ± 0.08^{ad}	4.80 ± 0.54^{abc}	1.76 ± 0.84^{ac}
30 days	Control (H_2O)	1.44 ± 0.30^{ac}	1.47 ± 0.09^{ad}	5.88 ± 1.18^{ac}	1.91 ± 0.09^{ac}
	Testosterone (4 mg/kg/3 days)	1.87 ± 0.12^{bc}	1.83 ± 0.37^{b}	5.22 ± 0.97^{abc}	1.93 ± 0.04^{ad}
	EGCMO 1 (1 mL/100 g)	1.34 ± 0.08^{a}	1.23 ± 0.05^{ce}	5.22 ± 0.49^{abc}	1.49 ± 0.27^{bc}
	EGCMO 2 (2 mL/100 g)	1.72 ± 0.38^{bc}	$1.\ 34 \pm 0.43^{ace}$	4.92 ± 0.87^{abc}	1.45 ± 0.40^{b}
	EGEMO 1 (1 mL/100 g)	1.82 ± 0.40^{bc}	1.38 ± 0.04^{acd}	5.72 ± 1.57^{abc}	1.77 ± 0.09^{ac}
	EGEMO 2 (2 mL/100 g)	1.88 ± 0.17^{bc}	1.43 ± 0.43^{ade}	4.92 ± 1.25^{abc}	1.69 ± 0.04^{c}
60 days	Control (H_2O)	1.81 ± 0.38^{bc}	1.53 ± 0.06^{d}	4.94 ± 1.01^{abc}	2.21 ± 0.13^{d}
	Testosterone (4 mg/kg/3 days)	1.91 ± 0.20^{b}	1.68 ± 0.17^{b}	5.78 ± 1.16^{ac}	2.16 ± 0.35^{d}
	EGCMO 1 (1 mL/100 g)	1.60 ± 0.30^{abc}	1.20 ± 0.015^{e}	4.95 ± 0.75^{abc}	1.65 ± 0.02^{b}
	EGCMO 2 (2 mL/100 g)	1.66 ± 0.33^{abc}	1.31 ± 0.13^{ace}	4.46 ± 0.58^{b}	1.58 ± 0.23^{b}
	EGEMO 1 (1 mL/100 g)	1.71 ± 0.45^{bc}	1.64 ± 0.08^{b}	5.78 ± 0.98^{c}	1.88 ± 0.062^{a}
	EGEMO 2 (2 mL/100 g)	$1.78 \pm 0.36^{\ bc}$	1.41 ± 0.12^{ad}	4.71 ± 0.58^{abc}	1.79 ± 0.094^{a}

Note: The data represented are the means ± SEM of the values of five animals. The different letters in superscript, a, b, c,… represent significant differences between the values of the various experimental groups at the threshold of $p < 0.05$ (Fisher LSD test).
Abbreviations: EGCMO, crude *Elaeis guineensis* mesocarp oil; EGEMO, emulsified *Elaeis guineensis* mesocarp oil.

HDL cholesterol significantly increased in all EGCMO- and EGEMO-treated groups, suggesting a cardioprotective effect, whereas testosterone had no significant impact.

LDL cholesterol decreased with both treatments, although EGCMO1 at 60 days showed relatively higher levels than controls, indicating dose- and time-dependent effects.

3.12 | Effects on Serum Oxidative Stress Parameters

Table 7 presents serum proteins, MDA, SOD, and CAT levels.

Total proteins increased significantly with EGCMO2 compared to EGEMO and testosterone groups, suggesting a protective effect on protein metabolism.

MDA levels decreased in all treated groups compared to controls, although EGCMO at certain doses increased MDA relative to EGEMO. Higher baseline MDA at day 0 suggests adaptive antioxidant responses over time.

SOD activity remained unchanged across all groups.

CAT activity increased significantly with EGCMO, particularly at higher doses and longer durations, indicating enhanced antioxidant defense.

3.13 | Effects on Testicular Oxidative Stress Parameters

Table 8 summarizes testicular biomarkers.

3.13.1 | Total Proteins

Testicular protein levels showed dose- and formulation-dependent variations, with EGEMO1 higher at low dose and EGCMO2 higher at high dose after 30 days.

3.13.2 | Cholesterol

EGEMO1 induced the highest cholesterol levels at 30 days, while EGCMO1 showed the highest levels at 60 days, suggesting accumulation with prolonged exposure.

3.13.3 | MDA

No significant variation in testicular MDA was observed across groups, indicating stable lipid peroxidation.

3.13.4 | SOD

EGCMO1 increased SOD at 30 days, whereas EGEMO1 showed the highest activity at 60 days. Overall, EGEMO groups exhibited higher SOD activity over time.

3.13.5 | CAT

CAT activity increased significantly in EGCMO1 and EGEMO2 at 30 days and in EGCMO2 and EGEMO2 at 60 days, indicating sustained antioxidant activation, particularly at higher doses.

4 | Discussion

The present study investigated the reproductive benefits of crude *Elaeis guineensis* oil (EGCMO) and its emulsified form (EGEMO) through a comprehensive evaluation of sperm parameters, hormonal balance, and antioxidant status in healthy Wistar rats. These experimental approaches were selected to elucidate the potential mechanisms through which palm oil-derived bioactive compounds enhance male fertility, particularly by modulating oxidative stress and improving testicular function. In parallel with these reproductive assessments, the study monitored body mass evolution and serum lipid profile to ensure that repeated oral administration of

TABLE 6 | Effect of treatment with *Elaeis guineensis* mesocarp oil and its emulsion on serum lipid parameters.

Treatment duration	Experimental group	Triglycerides (g/dL)	Total cholesterol (g/dL)	HDL cholesterol (g/dL)	LDL cholesterol (g/dL)
—	Day 0	1.91 ± 0.54^{ag}	0.61 ± 0.09^{adf}	0.15 ± 0.02^{abgh}	0.14 ± 0.04^{abd}
30 days	Control (H_2O)	0.63 ± 0.18^{b}	0.46 ± 0.05^{ab}	0.12 ± 0.01^{a}	0.21 ± 0.07^{a}
	Testosterone (4 mg/kg/3 days)	0.88 ± 0.25^{b}	0.46 ± 0.07^{a}	0.13 ± 0.03^{ah}	0.15 ± 0.04^{abd}
	EGCMO 1 (1 mL/100 g)	1.32 ± 0.20^{c}	0.68 ± 0.11^{abf}	0.19 ± 0.04^{begh}	0.22 ± 0.07^{a}
	EGCMO 2 (2 mL/100 g)	1.95 ± 0.29^{afg}	0.71 ± 0.02^{bdf}	0.25 ± 0.03^{cdei}	0.06 ± 0.02^{bd}
	EGEMO 1 (1 mL/100 g)	2.48 ± 0.07^{defghi}	1.38 ± 0.19^{c}	0.23 ± 0.04^{degi}	0.64 ± 0.24^{c}
	EGEMO 2 (2 mL/100 g)	1.96 ± 0.03^{afg}	0.68 ± 0.04^{adf}	0.22 ± 0.05^{egi}	0.06 ± 0.02^{bd}
60 days	Control (H_2O)	1.43 ± 0.24^{c}	0.50 ± 0.06^{ab}	0.13 ± 0.03^{a}	0.08 ± 0.03^{bd}
	Testosterone (4 mg/kg/3 days)	2.46 ± 0.15^{efghi}	0.70 ± 0.04^{df}	0.15 ± 0.01^{abegh}	0.07 ± 0.02^{bd}
	EGCMO 1 (1 mL/100 g)	2.26 ± 0.06^{fghi}	0.67 ± 0.03^{abdf}	0.18 ± 0.02^{begh}	0.03 ± 0.01^{d}
	EGCMO 2 (2 mL/100 g)	2.22 ± 0.44^{ghi}	0.74 ± 0.06^{df}	0.19 ± 0.03^{ghi}	0.10 ± 0.04^{abd}
	EGEMO 1 (1 mL/100 g)	2.46 ± 0.30^{hi}	1.97 ± 0.5^{e}	0.17 ± 0.04^{h}	1.29 ± 0.24^{e}
	EGEMO 2 (2 mL/100 g)	2.474 ± 0.18^{i}	0.81 ± 0.11^{f}	0.23 ± 0.02^{i}	0.08 ± 0.03^{bd}

Note: The data represented are the means ± SEM of the values of five animals. The different letters in superscript, a, b, c,… represent significant differences between the values of the various experimental groups at the threshold of $p < 0.05$ (Fisher LSD test).
Abbreviations: EGCMO, crude *Elaeis guineensis* mesocarp oil; EGEMO, emulsified *Elaeis guineensis* mesocarp oil.

TABLE 7 | Effect of EGCMO and EGEMO on serum oxidative stress parameters in treated rats.

Treatment duration	Experimental group	Serum proteins (g/dL)	Serum MDA (nmol/mg proteins)	Serum SOD (µmol/min/g proteins)	Serum CAT (mM H_2O_2/min/g proteins)
—	Day 0	0.75 ± 0.20^{a}	0.08 ± 0.01^{a}	2.69 ± 0.05^{ab}	2.86 ± 1.11^{a}
30 days	Control (H_2O)	0.76 ± 0.14^{a}	0.04 ± 0.01^{bd}	2.68 ± 0.08^{ab}	1.14 ± 0.46^{b}
	Testosterone (4 mg/kg/3 days)	0.57 ± 0.06^{b}	0.01 ± 0.00^{c}	2.63 ± 0.20^{ab}	1.40 ± 0.52^{b}
	EGCMO 1 (1 mL/100 g)	0.97 ± 0.03^{c}	0.03 ± 0.01^{bcd}	2.68 ± 0.14^{ab}	1.59 ± 0.38^{b}
	EGCMO 2 (2 mL/100 g)	1.10 ± 0.11^{d}	0.01 ± 0.00^{c}	2.54 ± 0.48^{ab}	1.67 ± 0.63^{b}
	EGEMO 1 (1 mL/100 g)	0.84 ± 0.15^{ac}	0.02 ± 0.00^{bc}	2.75 ± 0.06^{a}	2.49 ± 0.85^{a}
	EGEMO 2 (2 mL/100 g)	0.79 ± 0.15^{ac}	0.04 ± 0.02^{d}	2.77 ± 0.03^{a}	2.14 ± 1.01^{a}
60 days	Control (H_2O)	0.96 ± 0.19^{d}	0.06 ± 0.02^{e}	2.77 ± 0.04^{a}	0.71 ± 0.20^{c}
	Testosterone (4 mg/kg/3 days)	0.80 ± 0.06^{ad}	0.02 ± 0.00^{c}	2.54 ± 0.17^{ab}	2.32 ± 0.30^{a}
	EGCMO 1 (1 mL/100 g)	$0.75 \pm 0,03^{a}$	0.01 ± 0.00^{c}	2.45 ± 0.51^{b}	1.22 ± 0.51^{bc}
	EGCMO 2 (2 mL/100 g)	1.03 ± 0.08^{d}	0.02 ± 0.00^{bcd}	2.52 ± 0.25^{ab}	2.08 ± 0.74^{a}
	EGEMO 1 (1 mL/100 g)	0.84 ± 0.15^{ac}	0.04 ± 0.01^{bd}	2.79 ± 0.04^{a}	1.53 ± 0.59^{bc}
	EGEMO 2 (2 mL/100 g)	0.68 ± 0.03^{b}	0.03 ± 0.01^{bcd}	2.67 ± 0.11^{ab}	1.82 ± 0.51^{bc}

Note: The data represented are the means ± SEM of the values of five animals. The different letters in superscript, a, b, c,… represent significant differences between the values of the various experimental groups at the threshold of $p < 0.05$ (Fisher LSD test).
Abbreviations: CAT, catalase; EGCMO, crude *Elaeis guineensis* mesocarp oil; EGEMO, emulsified *Elaeis guineensis* mesocarp oil; MDA, malondialdehyde; SOD, superoxide dismutase.

TABLE 8 | Effect of EGCMO and EGEMO on testicular oxidative stress parameters.

Treatment duration	Experimental groups	Testicle proteins (µg/mg)	Testicle cholesterol (ng/mg)	Testicle MDA (nmol/mg proteins)	Testicle SOD (µmol/min/g proteins)	Testicle CAT (mM H_2O_2/min/g proteins)
—	Day 0	2.60 ± 0.06^{a}	33.97 ± 5.16^{a}	0.19 ± 0.00^{a}	8.98 ± 3.5^{ac}	1.28 ± 0.01^{a}
30 days	Control (H_2O)	1.20 ± 0.01^{bc}	20.87 ± 7.74^{b}	0.27 ± 0.02^{a}	23.88 ± 14.51^{ac}	1.17 ± 0.05^{a}
	Testosterone (4 mg/kg/3 days)	1.80 ± 0.08^{bf}	12.26 ± 5.66^{b}	0.22 ± 0.03^{a}	137.17 ± 60.37^{b}	1.96 ± 0.36^{b}
	EGCMO 1 (1 mL/100 g)	0.91 ± 0.02^{ce}	46.35 ± 1.79^{a}	0.29 ± 0.00^{a}	41.88 ± 26.11^{ad}	1.83 ± 0.37^{b}
	EGCMO 2 (2 mL/100 g)	3.42 ± 0.08^{d}	39.92 ± 19.22^{a}	0.13 ± 0.00^{a}	11.65 ± 4.23^{ac}	1.18 ± 0.07^{a}
	EGEMO 1 (1 mL/100 g)	2.73 ± 0.10^{a}	95.62 ± 8.76^{c}	0.12 ± 0.05^{a}	2.42 ± 0.06^{c}	1.24 ± 0.04^{a}
	EGEMO 2 (2 mL/100 g)	0.54 ± 0.00^{e}	42.29 ± 4.62^{a}	0.21 ± 0.00^{a}	69.46 ± 16.42^{de}	1.71 ± 0.24^{b}
60 days	Control (H_2O)	1.92 ± 0.03^{f}	20.14 ± 10.77^{b}	0.25 ± 0.03^{a}	39.73 ± 30.51^{ad}	0.90 ± 0.39^{a}
	Testosterone (4 mg/kg/3 days)	6.20 ± 0.03^{ce}	33.49 ± 1.98^{a}	0.28 ± 0.01^{a}	36.42 ± 13.66^{acd}	1.24 ± 0.07^{a}
	EGCMO 1 (1 mL/100 g)	1.21 ± 0.01^{bc}	58.61 ± 14.12^{d}	0.23 ± 0.04^{a}	48.08 ± 42.11^{d}	1.20 ± 0.43^{a}
	EGCMO 2 (2 mL/100 g)	2.48 ± 0.05^{af}	22.45 ± 12.20^{b}	0.23 ± 0.00^{a}	26.81 ± 7.48^{ac}	1.62 ± 0.71^{b}
	EGEMO 1 (1 mL/100 g)	1.69 ± 0.02^{b}	22.44 ± 12.19^{b}	0.21 ± 0.04^{a}	84.50 ± 40.52^{e}	1.26 ± 0.13^{a}
	EGEMO 2 (2 mL/100 g)	2.10 ± 0.02^{af}	22.40 ± 12.23^{b}	0.22 ± 0.04^{a}	40.75 ± 32.18^{ad}	1.59 ± 0.43^{b}

Note: The data represented are the means ± SEM of the values of five animals. The different letters in superscript, a, b, c,… represent significant differences between the values of the various experimental groups at the threshold of $p < 0.05$ (Fisher LSD test).

Abbreviations: CAT, catalase; EGCMO, crude *Elaeis guineensis* mesocarp oil; EGEMO, emulsified *Elaeis guineensis* mesocarp oil; MDA, malondialdehyde; SOD, superoxide dismutase.

EGCMO and EGEMO did not predispose animals to adverse metabolic outcomes. Since excessive lipid intake can promote weight gain, dyslipidemia, and increased cardiovascular risk, tracking these parameters served as a critical safety measure to verify that the fertility-enhancing benefits of palm oil would not be accompanied by tendencies toward obesity or cardiometabolic disturbances. The stability of both body mass and lipid indices across treatment groups, therefore, supports the notion that these palm oil formulations can confer reproductive benefits without compromising overall metabolic health. According to Loganathan et al. [20], red palm oil exhibits a wide range of pharmacological properties, including cardioprotective, antiatherogenic, antihemorrhagic, antihypertensive, anticancer, and antidiabetic activities. It has also been shown to improve ocular health, enhance chemotherapy outcomes, protect against hypobaric conditions and infections, and support normal reproductive function in both males and females.

The selected oil administered volume (1 mL) was based on previous studies by Ayeleso et al. [44], Naphatthalung et al. [45] and Kola-Ajibade et al. [46], which demonstrated that this administered dose is devoid of noted toxicological effects. The same dosage was applied in this study to reflect the approximate edible intake under prolonged daily consumption. A higher multiple of this dose (2 mL/100 g) was also tested to evaluate potential dose-dependent or adverse effects. It was hypothesized that crude palm oil would enhance male reproductive parameters and that its emulsified form would further potentiate these beneficial effects.

As part of this investigation, percentage weight changes, relative organ weights, and sperm parameters were measured in rats treated with varying doses of EGCMO and EGEMO. A significant reduction in testicular and seminal vesicle mass was observed in the reference group compared to the neutral controls. This decline, when contrasted with healthy controls, is likely due to the progestogenic and anti-androgenic effects of exogenous testosterone, which suppresses the growth of androgen-dependent organs. It is well established that the development and function of male reproductive organs are androgen-dependent [47]. The observed decreases in organ weights were consistent with reduced sperm production, motility, and daily sperm output.

Conversely, increased relative reproductive organ masses were noted in treated groups, particularly those receiving EGEMO. This enhancement may be attributed to the stimulation of reproductive hormones through the antioxidant capacity of palm oil, primarily mediated by bioactive components naturally found in palm oil such as vitamin E [20].

The weight gain observed in treated animals is likely linked to the high triglyceride content of crude palm oil, which comprises 95%–99% triglycerides [48], a key contributor to body fat. Moreover, recent nutritional studies suggest that the structural organization of fats, particularly in emulsified forms, significantly influences fat digestion and fatty acid bioavailability [49]. Palm oil and its emulsified form, as reported in this study, are rich in nutritionally valuable fatty acids, particularly palmitic and oleic acids, which were likely bioavailable to the treated animals and may have contributed to weight gain. Furthermore, the elevated serum and testicular cholesterol levels observed in EGEMO-treated animals may indicate enhanced intestinal and lymphatic absorption of fatty acids from the emulsified oil [47, 50].

Daily and effective sperm production significantly improved in groups treated with EGCMO and EGEMO1 at both 30 and 60 days, relative to both healthy and testosterone-treated controls. Histological analysis of testicular and epididymal tissues corroborated these findings, revealing no structural damage. Notably, sperm density in the seminiferous tubule lumen increased in a dose-dependent manner in EGEMO-treated rats, regardless of dose or treatment duration. These results reinforce the androgenic properties of EGCMO and its fertility-enhancing potential, consistent with the findings of Azubuike and Akomas [51], who showed that supplementation with EGCMO improved semen qualities in albino rats and supported testicular development.

Furthermore, daily and effective sperm production was significantly higher in the EGEMO1 group compared to the EGCMO1 group at equivalent time points. The suppressed parameters observed in controls are likely due to testosterone-induced inhibition of spermatogenesis, as testosterone regulates sperm production and transit via Sertoli cell-mediated mechanisms [52, 53]. The reduction observed in epididymal transit time compared to testosterone-treated animals further supports the protective effects of the treatments, as accelerated sperm transit shortens the time available for essential maturation processes, thereby compromising sperm development. Both EGCMO and EGEMO thus appear to preserve sperm maturation and enhance fertilizing potential.

PSA is commonly used as a biomarker for the early detection of prostate cancer. According to Hsieh et al. [54], elevated PSA levels are also associated with an increased risk of cardiovascular disease in men. In this study, administration of EGCMO and EGEMO resulted in reduced PSA levels, suggesting protective effects against both prostate cancer and cardiovascular disease. This protective role is further supported by improvements in serum lipid parameters following treatment.

Testosterone levels remained consistently higher in testosterone enanthate-treated animals throughout the treatment period. However, in palm oil-treated animals, testosterone levels were significantly elevated compared to controls after 30 days but declined after 60 days of administration. This biphasic pattern may reflect the dynamics of testicular activity. Since spermatogenesis in rats takes ~60 days, the oil may have stimulated a cycle of spermatogenesis early on, leading to feedback inhibition by the end of the cycle. This interpretation is supported by the observed reductions in FSH and LH levels in the palm oil-treated groups after 60 days of treatment.

Testosterone secretion is regulated by the hypothalamic–pituitary–gonadal (HPG) axis, which operates through a negative feedback mechanism. Once circulating testosterone reaches a certain threshold, it signals the hypothalamus and pituitary gland to inhibit GnRH and LH secretion, with FSH also being affected [55]. Palm oil contains bioactive secondary metabolites, including phenolic compounds and flavonoids, which may interact with androgen receptors in the hypothalamus or pituitary to modulate testosterone regulation and function [56]. The results of this study confirm that both EGCMO and EGEMO are particularly rich in these metabolites. Assessment of SOD, a key antioxidant enzyme and sperm quality marker, showed no significant difference across treatment groups. This is in line with Aboua et al. [27], who reported that red palm oil did not influence oxidative enzyme activity. Since SOD is expressed in Sertoli cells and localized in the epididymis, the results suggest that neither EGCMO nor EGEMO altered endogenous

antioxidant enzyme expression. Similarly, MDA levels remained unchanged despite its presence in palm oil [57], indicating no induced lipid peroxidation. As indicated by the GC-FID analysis, the omega-6/omega-3 ratio in the emulsified form of the oil (30) was slightly lower than that of EGCMO (33.33). This reduction may lessen the oil's pro-inflammatory effects and enhance its anti-inflammatory potential [58], as reflected by the improved antioxidant capacity observed in the emulsified oil.

Comparative analysis of EGCMO and EGEMO treatments revealed greater testicular and seminal vesicle masses in the EGEMO1 group. This is supported by enhanced sperm output, motility, and shortened epididymal transit time. The superior results associated with EGEMO can be attributed to its emulsified structure, which allows better dispersion into small droplets, enhancing the bioavailability of antioxidant and phenolic constituents [59].

Histological examinations provided further support, with improved organization of seminiferous tubules in EGEMO1-treated rats after 30 days of treatment. This structural enhancement coincided with higher testosterone levels (1.82 ± 0.40) compared to those treated with EGCMO1 (1.34 ± 0.08). Although cholesterol levels were also elevated in EGEMO-treated animals, this may reflect more efficient cholesterol absorption, considering cholesterol's role as a precursor in steroid hormone biosynthesis [60]. By day 60, a reversal in cholesterol levels was noted, with EGEMO-treated rats showing lower values than EGCMO-treated ones, indicating normalization over time.

In terms of cardiovascular implications, the lipid profile of EGEMO-treated animals showed increased total cholesterol, HDL, and triglycerides, with no significant change in LDL levels. These changes suggest enhanced lipid absorption due to emulsification. This finding is supported by recent literature on fat structure and bioavailability [61] and the known composition of palm oil, which is ~98% triglycerides [62]. The elevation in HDL levels aligns with a study by Hisham et al. [63], who reported increases in HDL and LDL with palm oil intake. Importantly, the lipid profiles did not indicate any risk of hypercholesterolemia or cardiovascular dysfunction in treated animals.

This study confirms that crude palm oil enhances male reproductive functions in rats, notably sperm production and testicular integrity, primarily through the action of secondary metabolites rather than endogenous hormone stimulation. Emulsified palm oil demonstrated superior efficacy, likely due to enhanced bioavailability of active compounds. These findings suggest that emulsification may improve the therapeutic value and acceptability of palm oil. Unlike exogenous testosterone, which disrupts the reproductive axis via negative feedback inhibition, palm oil and its emulsion appear to support fertility without adverse hormonal consequences. Testosterone negatively regulates its own production by inhibiting GnRH, LH, and FSH release [64], ultimately suppressing spermatogenesis. In contrast, palm oil-based treatments offer a natural and potentially safer alternative for improving male fertility while maintaining cardiovascular health.

Crude palm oil exhibits a direct beneficial effect on sperm parameters; however, its efficacy is significantly enhanced when administered in emulsified form. Treatment with emulsified palm oil resulted in elevated serum hormone levels, which in turn stimulated spermatogenesis and contributed to the amelioration of male infertility. Histological analyses demonstrated reorganization of the seminiferous tubule architecture, characterized by increased tubular diameter and thickening of the germinal epithelium, findings that support the androgenic activity of the emulsified preparation. Furthermore, emulsification markedly improved the antioxidant potential and phenolic content of the oil, enhancing its capacity to counteract oxidative stress. These results indicate that palm oil emulsion may serve as a protective agent for sperm integrity and a promising strategy for supporting male reproductive health.

A key limitation of this study is the use of a healthy animal model rather than one with induced reproductive pathology. Although this design restricts conclusions about therapeutic efficacy in disease states, it was intentionally selected to elucidate the intrinsic mechanisms through which EGCMO and EGEMO influence reproductive physiology, oxidative balance, and spermatogenesis. Establishing these baseline mechanistic effects is essential before assessing their curative potential under pathological conditions. Another limitation is the absence of in-depth molecular investigations, particularly the evaluation of fertility-related gene expression. Future studies should examine key genes involved in spermatogenesis (e.g., DAZL, STRA8, and c-KIT) and steroidogenesis (e.g., STAR, CYP11A1 and HSD3B1) [65] to better define the pathways modulated by EGCMO and EGEMO. Furthermore, testing these formulations in pathological models such as cyclophosphamide- or cadmium-induced testicular toxicity or metabolic dysfunction models like high-fat diet-induced obesity or streptozotocin-induced diabetes [66] would help determine their efficacy under clinically relevant conditions. Such investigations would substantially strengthen the translational value of this work and confirm the phytotherapeutic potential of EGCMO and EGEMO in managing male infertility.

Author Contributions

Silviane Kenne Foppa: investigation, software, writing – original draft, resources, writing – review and editing. **Landry Lienou Lienou**: conceptualization, investigation, resources, reviewing, software, visualization. **Modeste Wankeu-Nya**: methodology, investigation, supervision, data curation. **Fabrice Fabien Dongmo Dongho**: methodology, supervision, data curation, reviewing. **Laeticia Ade Sah-Koh, Christian Claude Ntep, and Michelle Maeva Monthe Niyou**: methodology, investigation. **Rebecca Madeleine Etame Ebelle**: supervision, reviewing, project administration. **Maria Helena Tavares De Matos and Ana Paula Ribeiro Rodrigues**: supervision, reviewing. **Phélix Bruno Telefo**: conceptualization, supervision, reviewing.

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All the authors contributed to the final version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data are available from the corresponding author upon appropriate request.

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