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Cocos nucifera L. oil alleviates lead acetate-induced reproductive toxicity in sexually-matured male Wistar rats

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Abstract

Objectives: Lead primarily affects male reproductive functions via hormonal imbalance and morphological damage to the testicular tissue with significant alteration in sperm profile and oxidative markers. Though, different studies have reported that *Cocos nucifera L.* oil has a wide range of biological effects, this study aimed at investigating the effect of *Cocos nucifera L.* oil on lead acetate-induced reproductive toxicity in male Wistar rats.

Methods: Twenty (20) sexually matured male Wistar rats (55–65 days) were randomly distributed into four groups (n=5). Group I (negative control)—distilled water orally for 56 days, Group II (positive control)—5 mg/kg bwt lead acetate intraperitoneally *(i.p.)* for 14 days, Group III—6.7 mL/kg bwt *Cocos nucifera L.* oil orally for 56 days and Group IV—lead acetate intraperitoneally *(i.p.)* for 14 days and *Cocos nucifera L.* oil for orally for 56 days. Rats were sacrificed by diethyl ether, after which the serum, testis and epididymis were collected and used for semen analysis, biochemical and histological analysis.

Results: The lead acetate significantly increases ($p<0.05$) testicular and epididymal malondialdehyde (MDA) levels, while a significant reduction ($p<0.05$) in sperm parameters, organ weight, testosterone and luteinizing hormone was observed when compared with the negative control. The coadministration of *Cocos nucifera* oil with lead acetate significantly increases ($p<0.05$) testosterone, luteinizing hormone, sperm parameters and organ weight, with a significant decrease ($p<0.05$) in MDA levels compared with positive control. Histological analysis showed that lead acetate distorts testicular cytoarchitecture and germ cell integrity while this was normalized in the cotreated group.

Conclusions: *Cocos nucifera* oil attenuates the deleterious effects of lead acetate in male Wistar rats, which could be attributed to its polyphenol content and antioxidant properties.

Keywords: *Cocos nucifera* oil; lead acetate; reproductive system; Wistar rats.

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Introduction

There is a growing concern about the link between environmental exposure to heavy metals and the effects on the male reproductive system [1]. Individuals are exposed to toxic metals like lead, cadmium, and mercury through the intake of contaminated food and water [2]. Lead is a heavy metal increasingly present in our environment, especially in Nigeria, due to rapid industrialization and poor waste management system. Individuals working in and dealing with the motor vehicle assembly, panel beating, battery making, soldering, lead excavation and smelting, glass, plastics, printing, ceramics, and paint industries are on

many occasions exposed to lead [3]. Exposure to lead in humans occurs mainly through ingestion via the respiratory and gastrointestinal systems which are eventually stored in soft tissue and bones [4]. However, lead toxicity on the male reproductive system has been a major source of concern and research interest for several years; it is an important part of idiopathic infertility owing to the growing decline in sperm parameters such as motility, counts, and viability. The direct effects of lead toxicity on the male reproductive system have been linked to the involvement of multiple pathways such as direct toxicity on sperm cell and disruption in the hypothalamic–pituitary–gonadal axis of endocrine functions [5]. Studies have revealed that the adverse effects of lead on sperm cells may be partially reversible at a very mild concentration. However, accumulation of this toxicant will lead to total degeneration of testicular organs due to apoptosis, necrosis and hormonal imbalance, according to Viskum et al. [6].

Traditional medicine across the globe has utilized coconut in the treatment of a wide variety of health problems [7]. It has been reported to be used as anthelmintic, antidotal, antiseptic, astringent, bactericidal, diuretic, purgative and anti-convulsant [8]. Coconut oil, a major ketogenic diet, has important interest mainly because it possesses therapeutic properties than any other dietary oil and has a wide range of health benefits [9]. Coconut oil is known to compose essentially a special group of fat molecules referred to as medium-chain fatty acids (MCFA). It contains about 92% saturated fatty acids which are mostly medium-chain triglycerides made up of lauric acid (antimicrobial properties), caprylic acid (fungicidal), capric acid, palmitic acid and myristic acid utilized for boosting the immune system, reducing prostate enlargement and cancer treatment [9]. Though several literature works have reported the use of *Cocos nucifera L.* oil in a wide range of biological effects, there is a paucity of data on its use as a pro-fertility agent. This study was designed to provide further elucidations on the effect of *Cocos nucifera L.* oil on lead acetate-induced male reproductive toxicity in male Wistar rats.

Materials and methods

Collection of the plant material

Twenty coconuts were purchased from a local market in Kuje, Abuja, Nigeria, and it was authenticated and identified at the Department of Biological Sciences, Bingham University, Karu, with a collector's specimen number LJEN 149 and herbarium number 154.

Preparation of the *Cocos nucifera L.* Oil

The coconut oil was extracted using a modified wet extraction method, as previously described by [10]. Briefly, the solid endosperm of the coconut was crushed and made into a viscous slurry. The viscous slurry obtained was squeezed through a fine sieve to obtain coconut milk and 500 ml of water was added. The concomitant coconut milk was left for about 24 h to facilitate the gravitational separation of the emulsion [11]. Demulsification produced layers of an aqueous phase (water) on the bottom, an emulsion phase (cream) in the middle layer and an oil phase on top [12]. The oil on top was scooped and heated for about 5 min to remove moisture. The obtained coconut oil was filtered through a fine sieve and stored at room temperature until when required.

Experimental animals

Twenty (20) sexually matured male Wistar rats (55–65 days) were obtained from the National Veterinary Research Institute, Vom, Plateau State, for this experiment. The rats were kept in the Bingham University animal care unit and allowed to acclimatize for two weeks before the experiments' commencement. The rats were fed with standard rat chow and water *ad libitum*. The experiments complied with the guidelines of the institution's animal ethics committee, which was established under the internationally accepted principles for laboratory animal use and care.

Experimental design

The animals were randomly divided into four groups (n=5) and administered the following doses of substances.

Group 1: Animals served as the negative control group and received 10 mL/kg body weight (bwt) distilled water orally for 56 days.

Group 2: Animals were treated with 5 mg/kg bwt lead acetate intraperitoneally *(i.p.)* daily for 14 days as the positive control.

Group 3: Animals were treated with 6.7 mL/kg bwt *Cocos nucifera L.* oil orally daily for 56 days.

Group 4: Animals were treated 5 mg/kg bwt lead acetate (*i.p.)* daily for 14 days plus 6.7 mL/kg body weight, *Cocos nucifera L.* oil orally daily for 56 days.

After the last day of administration, rats were placed in a gas anesthetic chamber and euthanized under Isofor anesthesia (100 mg/kg bwt). *A*fterward, approximately 5 mL of blood was collected from the animals via cardiac puncture into different sterile tubes for reproductive hormonal assay, and rats were sacrificed through diethyl ether anesthesia. Testes and epididymis were collected and used for semen analysis, histology, and malondialdehyde (MDA) activity. Other organs, seminal vesicle and prostate gland were also collected.

Experimental protocols

Malondialdehyde (MDA) level: MDA, a biomarker of lipid peroxidation, was determined in the testicular and epididymal homogenates by measuring the thiobarbituric acid reactive substances (TBARS) produced during the lipid peroxidation. This method was described by [13], based on the reaction between 2-thiobarbituric acid (TBA) and malondialdehyde: an end product of lipid peroxidation. Briefly, 50 mg of tissues were

homogenized in 0.2% phosphoric acid (450 µL), centrifuged at 10,000 rpm for 10 min to collect the supernatant. H_3PO_4 (1%, 3 mL) and TBA (0.6%, 1 mL) were mixed with 500 µl of the homogenates, vortexed and heated for 45 min in a boiling water bath and allowed to cool. After that, 400 µL of *n*-butanol was added, vortexed to ensure vigorous mixing and centrifuge to separate the mixture into two different layers. The top (butanol) layer was transferred to a microplate, and absorbance was read at 535 and 520 nm. TBA concentration was obtained by finding the difference in the absorbance values.

Determination of testosterone, follicle-stimulating hormone and luteinizing hormone: The concentrations of testosterone, follicle-stimulating hormone and luteinizing hormone in the serum samples were determined through a tube-based enzyme immunoassay (EIA) technique. The protocol used in hormone testing followed the technique described by the kit manufacturers (Immunometrics Limited UK) and met the WHO research program standards for reproductive studies. The absorbance value for each sample and standard was measured at 450 ± 2 nm using a microplate reader (Jenway 6300 spectrophotometer, Essex, UK).

Semen analysis

The caudal epididymis of the right testis was isolated and lacerated to obtain semen used the semen used for sperm profiling as reported by [14].

Sperm motility: Two drops of sperm suspensions obtained from the caudal epididymis and placed on the surface of a prewarmed microscope slide; 2.9% sodium citrate (two drops) was added and covered with a coverslip. The number of progressively motile cells was recorded and divided by the total number of spermatozoa counted and expressed in percentage. About 25 fields of view were examined under a light microscope at 40× magnification [15].

Sperm count: The epididymal sperm samples were obtained from a known weight of *Cauda epididymis* into physiological saline in the ratio of 1:10 wt by volume. After vigorous pipetting to release the sperm cells, epididymal sperm count was assessed using the improved Neubauer hemocytometer and sperm cells in five Thoma chambers were counted. Sperm count was expressed as a million/mL of suspension [15].

Sperm viability: The sperm viability test was conducted from the suspension obtained from the caudal epididymis using the "Eosin–Nigrosin one-step staining technique." A portion of the sperm suspension was mixed with an equal volume of Eosin–Nigrosin stain and five air-dried smears were prepared on glass slides for each sample. The slides were examined within 15 min for percentage viability. Normal live sperm cells excluded the stain and appeared whitish, whereas dead sperm cells took up the stain and appeared pinkish. Percentage viability was calculated based on the number of live sperm cells out of the total number of sperm cells observed.

Histological analysis

The left testes were fixed in bouin's fluid for 48 h and were dehydrated in four concentrations of Isopropyl alcohol, i.e., 70, 80, 90, 100% for 1 h each, and then cleared in xylene before embedded in molten paraffin wax to remove the isopropyl alcohol. Serial sections (2 per animals) of 5 µm thick were done using Leica RM 212 Rt Rotary Microtome and tissues were stained using haematoxylin and eosin to demonstrate general tissue structure. A drop of mountant was placed on the slide's surface and covered with a 22 by 22 cm coverslip. Light microscopy was used for the evaluations, and photomicrographs were taken. Afterward, tissue pathology was evaluated by a pathologist blinded to the study.

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM) and analyzed using Analysis of variance (ANOVA) Graphpad prism software version 5.1. This was followed by Tukey post-hoc test to determine the level of significance between and within groups. A value of $p<0.05$ was considered statistically significant.

Results

Effect of *Cocos nucifera* oil on relative organ weight for 56 days in lead acetate-induced toxicity in Wistar rats

The results in Table 1 showed that the lead acetate treated group caused a significant decrease in testis, epididymal, seminal vesicle, and prostate weight when compared with the control. The group cotreated with lead acetate plus *Cocos nucifera* oil showed a slight increase in seminal vesicle weight and a significant increase in testis, epididymal and prostate weight as compared to the group treated with lead acetate only.

Effect of *Cocos nucifera* oil on testicular and epididymal malondialdehyde (MDA) levels for 56 days in lead acetate-induced toxicity in Wistar rats

The results in Table 2 showed that the lead acetate treated group had a significant increase in testicular and epididymal malondialdehyde (MDA) levels as compared to the control. The group cotreated with lead acetate plus *Cocos nucifera* oil showed a significant reduction in testicular and epididymal MDA levels as compared to the lead acetate treated group only.

Table 1: Effect of *Cocos nucifera* oil on relative organ weight for 56 days in lead acetate-induced toxicity in Wistar rats.

Weight, g	Group-1	Group-2	Group-3	Group-4
Testis	1.05 ± 0.16	0.92 ± 0.10[a]	1.22 ± 0.06	1.16 ± 0.08[b]
Epididymis	0.49 ± 0.06	0.31 ± 0.04[a]	0.58 ± 0.05	0.58 ± 0.02[b]
Seminal vesicle	0.91 ± 0.06	0.37 ± 0.07[aa]	0.58 ± 0.05	0.42 ± 0.04
Prostate	0.52 ± 0.00	0.26 ± 0.02[a]	0.30 ± 0.03	0.41 ± 0.03[bb]

Values are represented by mean ± SEM (n=5). [a]p<0.05, [aa]p<0.01 vs. control (group-1) while [b]p<0.05, [bb]p<0.01 vs. lead acetate (group-2).

Table 2: Effects of *Cocos nucifera* oil on testicular and epididymal malondialdehyde levels for 56 days in lead acetate-induced toxicity in Wistar rats.

MDA, nmol/mg protein	Group-1	Group-2	Group-3	Group-4
Testis	1.76 ± 0.27	8.05 ± 1.13[a]	2.54 ± 0.11	4.08 ± 1.86[b]
Epididymis	17.63 ± 28.1.16	28.76 ± 1.16[aa]	8.11 ± 1.99	7.40 ± 1.16[bb]

Values are represented by mean ± SEM (n=5). [a]p<0.05, [aa]p<0.01 vs. control (group-1) while [b]p<0.05, [bb]p<0.01 vs. lead acetate (group-2).

Effect of *Cocos nucifera* oil on hormonal levels for 56 days in lead acetate-induced toxicity in Wistar rats

The results in Table 3 showed that the lead acetate treated group had a significant decrease in the hormonal levels as compared to the control. The group cotreated with lead acetate plus *Cocos nucifera* oil showed a significant increase in testosterone and LH levels as compared to the group treated with lead acetate only.

Effect of *Cocos nucifera* oil on semen analysis for 56 days in lead acetate-induced toxicity in Wistar rats

Table 4 showed that lead acetate treated group caused a significant decrease sperm parameter with an increased number of nonviable sperm cells compared to the control. The group cotreated with lead acetate plus *Cocos nucifera* oil showed a significant increase in sperm concentration, motility and viability as compared to the group treated with lead acetate only.

Effect of *Cocos nucifera* oil on testicular tissue histology for 56 days in lead acetate-induced toxicity in Wistar rats

The testicular section from the negative control group (1A) showed normal testicular histoarchitecture with normal appearance of seminiferous tubules (ST), elongating spermatids (ES), Sertoli cells (red arrow) and Leydig cells (green arrow).

The testicular section from the positive control group (1B) showed significant distortion in the seminiferous tubules. The seminiferous tubules (ST) regress and atrophy, with the cessation of germ cells and cellular degeneration at various stages of spermatogenesis. The interstitial space (IC) became wider with atrophy of the Leydig cells.

The testicular section from *Cocos nucifera* only group (1C) showed testicular architecture similar to negative control with normal appearance of the seminiferous tubules (ST), growth of the germinal epithelium and elongated spermatids. The interstitial space and Leydig cells (green arrow) appear normal.

The testicular section from lead acetate and *Cocos nucifera* (1D) showed mild distortion in the seminiferous tubule. The tubule (ST) appears normal with few

Table 3: Effect of *Cocos nucifera* oil on hormonal levels for 56 days in lead acetate-induced toxicity in Wistar rats.

Hormones	Group-1	Group-2	Group-3	Group-4
Testosterone, ng/mL	0.81 ± 0.13	0.40 ± 0.04[a]	2.01 ± 0.77	0.76 ± 0.06[b]
FSH, mIU/mL	0.29 ± 0.03	0.22 ± 0.02	0.28 ± 0.02	0.27 ± 0.04
LH, mIU/mL	0.95 ± 0.02	0.29 ± 0.02[a]	0.86 ± 0.01	0.91 ± 0.06[b]

Values are represented by mean ± SEM (n=5). [a]p<0.05 vs. control (group-1) while [b]p<0.05 vs. lead acetate (group-2).

Table 4: Effect of *Cocos nucifera* oil on semen analysis for 56 days in lead acetate-induced toxicity in Wistar rats.

Sperm parameter	Group-1	Group-2	Group-3	Group-4
Motility, %	64.00 ± 4.00	49.00 ± 3.74[a]	76.00 ± 8.71	60.00 ± 5.47[b]
Sperm conc. (100,000/mL)	61.20 ± 3.72	53.80 ± 3.30[a]	84.00 ± 1.26	67.80 ± 5.85[b]
Viable cell, %	68.00 ± 4.89	48.00 ± 3.74[a]	78.00 ± 4.89	64.00 ± 4.00[b]
Non-viable cells, %	28.00 ± 14.40	30.00 ± 21.22	20.00 ± 11.22	40.00 ± 4.00[b]

Values are represented by mean ± SEM (n=5). [a]p<0.05 vs. control (group-1) while [b]p<0.05 vs. lead acetate (group-2).

elongating spermatids and well-arranged Sertoli cells (red arrow). However, there is a degeneration of Leydig cells in the interstitial space (Figure 1).

Discussion

There are accumulating evidence showing that lead exposure imparts negatively on male reproduction with significant alterations in both structure and functions of reproductive organs. Lead exposure promotes oxidative damage and inflammation of testicular cells, thereby disrupting endocrine and spermatogenic functions. Studies have shown that the weights of the reproductive organs are an indication of the capacity of its functions [16]. This study leads acetate-reduced testicular, epididymal, seminal vesicle and prostate weight, resulting from loss of tissue protein and cellular degeneration in the reproductive tissue due to increased generation of reactive oxygen species overwhelm the antioxidant defense system [17]. The increased organ weight observed in the cotreated group might be caused by fatty acid contents in *Cocos nucifera* oil, thus increasing the reproductive organ's weight due to the accumulation of fatty acid contents of *Cocos nucifera* oil.

Mason et al. [5] have shown that the detrimental role of lead acetate on the male reproductive system may be mediated via several pathways leading to the generation of free radicals and cellular damage. From our results, we observed that the group treated with lead acetate showed a significant increase in both testicular and epididymal MDA levels. The findings are in line with the work of Dorostghoal et al. [18], which showed that lead acetate increased reactive oxygen species in the male reproductive organ. However, coadministration with *Cocos nucifera* oil reduced

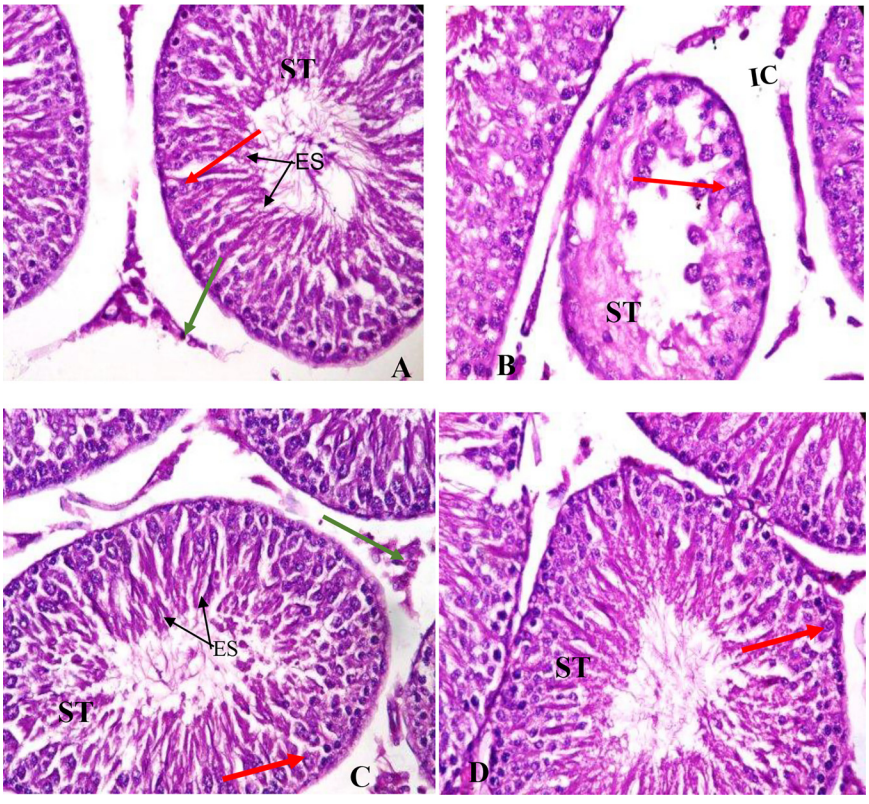


Figure 1: The photomicrograph of the testis in the negative control rat (A), positive control (B), *Cocos nucifera* only (C) and lead-acetate plus *Cocos nucifera* (D) rats. H&E stain, x400, scale bar 15 µm.

MDA levels. This suggests that *Cocos nucifera* oil may contain bioactive constituents with antioxidant properties capable of reducing lipid peroxidation [10].

The findings from hormonal assay analysis suggest that the significant increase in the LH level observed in the *Cocos nucifera* oil group may enhance testosterone production from the Leydig cells, which is essential for spermatogenesis [19]. The effects of *Cocos nucifera* oil on reproductive hormones may be due to an increase in testicular volume, testicular nutritional status, and increased testicular antioxidant contents. Cholesterol is known to be the primary source of testosterone synthesis; thus, *Cocos nucifera* oil possesses a high level of fatty acid, which may enhance the increased production of testosterone noticed in the group treated with lead acetate plus *Cocos nucifera* oil.

Our semen analysis results revealed that the lead acetate treated group demonstrated a significant decrease in all the semen parameters measured with an increase in non-viable sperm cells. This may be due to the disruption of the reproductive hormones, increased level of lipid peroxidation, reduction in testicular weight due to seminiferous tubules atrophy, and cessation of germ cells maturation, ultimately affecting the quantity of sperm production, which is in line with work done by Dorostghoal et al. [18]. The adverse effects of lead acetate on sperm characteristics seem to be at least reversed with *Cocos nucifera* oil treatment when administered simultaneously. These effects may be attributed to the presence of a high amount of fatty acids, such as lauric acid, caprylic acid, capric acid, myristic, and palmitic acid in *Cocos nucifera* oil. Studies have shown that the testicular sperm cell and the epididymis contain a high content of fatty acids like myristic, palmitic, caproic, caprylic, and capric acid, which may be responsible for the structural integrity of the plasma membrane required for lowering lipid peroxidation damage and enhancing fertility level [20]. Daniel et al. [21] reported that the epididymal fluid contains some fatty acids that may act as a signaling molecule and regulator of lipid secretion by the epididymal cells. It is known that lipids are a source of substrate for spermatozoa during motility and maturation. Thus, the effects seen by treatment with *Cocos nucifera* oil could be attributed to the fatty acid contents and antioxidant contents, thereby boosting the spermatogenesis and steroidogenic activity of the testis.

As seen in plates A-D, the morphological analysis indicates that *Cocos nucifera* oil may prevent structural damage to the testicular histoarchitecture by enhancing the seminiferous tubules' integrity and supporting Sertoli cells. Thereby ensuring germinal epithelial growth as well as the proliferation of sperm cells at various stages. These findings support the antioxidant effect of *Cocos nucifera* oil as stated by Nevin and Rajamohan [10] and the report of Fischer et al. [20], which showed that *Cocos nucifera* oil administration could cause testicular cell proliferation, development seminiferous tubule epithelium and concluded that it could lead to increased spermatogenesis and steroidogenesis

Conclusion

Cocos Nucifera oil attenuates the deleterious effects of lead acetate-induced reproductive toxicity in male Wistar rats. These effects could be attributed to the active constituents and antioxidant properties of *Cocos Nucifera* oil.

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References

1. Pasqualotto FF, Lucon AM, Sobreiro BP, Pasqualotto EB, Arap S. Effects of medical therapy, alcohol, smoking, and endocrine disruptors on male infertility. Rev Hosp Clin Fac Med Sao Paulo 2004;59:375–82.
2. Ezekwesili-Ofili J, Okaka A. Herbal medicines in African Traditional medicine; 2019. Available from: https://www.intechopen.com/books/herbal-medicine/herbal-medicines-in-african-traditional-medicine.
3. Kiziler AR, Aydemir B, Onaran I, Alici B, Ozkara H, Gulyasar T, et al. High levels of cadmium and lead in seminal fluid and blood of smoking men are associated with high oxidative stress and damage in infertile subjects. Biol Trace Elem Res 2007;120:82–91.
4. Mudipalli A. Lead hepatotoxicity & potential health effects. Indian J Med Res 2007;126:518–27.
5. Al-Omary HL, Alawad ZM, Jaafar I. Environmental lead exposure and male infertility. IOSR J Dent Med Sci (IOSR-JDMS) 2016;15:49–54.

6. Viskum S, Rabjerg L, Jorgensen PJ, Grandjean P. Improvement in semen quality associated with decreasing occupational lead exposure. Am J Ind Med 1999;35:257–63.
7. Kelly J, Kaufman D, Kelley K, Rosenberg L, Anderson T, Mitchell A. Recent trends in use of herbal and other natural products. JAMA Intern Med 2005;165:281–6.
8. Meraiyebu A, Olaniyan O, Oshimakinde A, Adinoyi S, Okere S. The role of coconut oil on Pentylenetetrazole induced convulsion in rats: an electroencephalogram study. Br J Pharmaceut Res 2014; 4:862–70.
9. Kossoff EH, Hartman AL. Ketogenic diets: new advances for metabolism-based therapies. Curr Opin Neurol 2012;25:173–8.
10. Nevin KG, Rajamohan T. Beneficial effects of virgin coconut oil on lipid parameters and in vitro LDL oxidation. Clin Biochem 2004; 37:830–5.
11. Onsaard E, Vittayanont M, Srigam S, McClements DJ. Properties and stability of oil-in-water emulsions stabilized by coconut skim milk proteins. J Agric Food Chem 2005;53:5747–53.
12. Nour A, Mohammed F, Yunus R, Arman A. Demulsification of virgin coconut oil by centrifugation method: a feasibility study. Int J Chem Tech 2009;1:59–64.
13. Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 1978;86:271–8.
14. Raji Y, Udoh US, Mewoyeka OO, Ononye FC, Bolarinwa AF. Implication of reproductive endocrine malfunction in male antifertility efficacy of Azadirachta indica extract in rats. Afr J Med Med Sci 2003;32:159–65.
15. Badkoobeh P, Parivar K, Kalantar SM, Hosseini SD, Salabat A. Effect of nano-zinc oxide on doxorubicin- induced oxidative stress and sperm disorders in adult male Wistar rats. Iran J Reprod Med 2013;11:355–64.
16. Mahmoud A, Goemaere S, El-Garem Y, Van-Pottelbergh I, Comhaire F, Kaufman J. Testicular volume in relation to hormonal indices of Gonadal function in community-dwelling elderly men. J Clin Endocrinol Metab 2003;88:179–84.
17. Asadi N, Bahmani M, Kheradmand A, Rafieian-Kopaei M. The impact of oxidative stress on testicular function and the role of antioxidants in improving it: a review. J Clin Diagn Res: JCDR 2017; 11:Ie01–5.
18. Dorostghoal M, Seyyednejad SM, Jabari A. Protective effects of Fumaria parviflora L. on lead-induced testicular toxicity in male rats. Andrologia 2014;46:437–46.
19. van Geel TA, Geusens PP, Winkens B, Sels JP, Dinant GJ. Measures of bioavailable serum testosterone and estradiol and their relationships with muscle mass, muscle strength and bone mineral density in postmenopausal women: a cross-sectional study. Eur J Endocrinol 2009;160:681–7.
20. Fischer V, Francis C, Fischer C. Micro architecture of the testes following administration of coconut oil (Cocos nucifera L.) on adult Albino Wistar rats. IOSR J Pharm Biol Sci 2019;14:01–9.
21. Ramos Angrimani DS, Nichi M, Losano JDA, Lucio CF, Lima Veiga GA, Franco MVMJ, et al. Fatty acid content in epididymal fluid and spermatozoa during sperm maturation in dogs. J Anim Sci Biotechnol 2017;8:18.