NJBMB (2022) 37(3) 222-228

Nigerian Journal of Biochemistry and Molecular Biology

The Official Publication of the Nigerian Society of Biochemistry & Molecular Biology (NSBMB). Journal homepage: https://www.nsbmb.org.ng/journals

e-ISSN: 2659-0042

Research Article

The Effects of Quercetin on Testicular Toxicity Induced by Water-soluble Fraction of Crude Oil in Male Wistar Rats

Emeka W. Ugwuishi[1], Onome B. Oghenetega[2*], Maduka L. Nweke[3], Obukohwo M. Oyovwi[4], Gloria E. Oghenetega[5], Victor Emojevwe[6], Patrick G. Okwute[2], Tunde F. Abraham[7]

[1]*Department of Physiology, College of Medicine, Enugu State University, Enugu, Nigeria*
[2]*Department of Physiology, School of Basic Medical Sciences, Babcock University, Ilishan-Remo, Nigeria*
[3]*Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, University of Nigeria, Nsukka, Nigeria*
[4]*Department of Hunan Physiology, Achievers University, Owo, Nigeria*
[5]*Department of Biochemistry, Faculty of Science, Delta State University, Abraka Nigeria*
[6]*Department of Physiology, University of Medical Sciences, Ondo, Nigeria*
[7]*Department of Physiology, Faculty of Basic Medical Sciences, University of Ibadan, Ibadan, Nigeria*

OPEN ACCESS

CORRESPONDENCE
Oghenetega O. B.
tegabonome@gmail.com
+234-816-514-8214

ARTICLE HISTORY
Received: 06/07/2022
Reviewed: 23/08/2022
Revised: 06/09/2022
Accepted: 09/10/2022
Published: 29/10/2022

CITATION
Ugwuishi, E. W., Oghenetega, O. B., Nweke, M. L., Oyovwi, O. M., Oghenetega, G. E., Emojevwe, V., Okwute, P. G. and Abraham, T. F. (2022). The Effects of Quercetin on Testicular Toxicity Induced by Water-soluble Fraction of Crude Oil in Male Wistar Rats. *Nigerian Journal of Biochemistry and Molecular Biology*. 37(3), 222-228

ABSTRACT

Crude oil has been reported to have adverse biological effects that are attributable to the dissolved low molecular weight hydrocarbons and metallic ions which are the major components of the water-soluble fraction of crude oil. Although, the protective effect of quercetin on testicular toxicity induced by the ingestion of Nigerian Bonny light crude oil has been reported. This study investigated the protective effects of quercetin on testicular toxicity induced by the water-soluble fraction of Nigerian Bonny light crude oil in male Wistar rats. Twenty (20) healthy male Wistar rats weighing about 100-120g were randomly selected into four major groups of five animals each. Animals were gavage daily with 1ml of distilled water (control), 1ml of 50% of water-soluble fraction of crude oil and quercetin at a dose of 10 mg/kg body weight for six (6) weeks. Serum testosterone level significantly decreased in male Wistar rats treated with water-soluble fraction of crude oil when compared to control ($P<0.001$). Moreover, water-soluble fraction of crude oil significantly increase testicular malondialdehyde ($P<0.001$), IL-1beta ($p<0.01$) and reduces superoxide dismutase ($p<0.01$), Zinc ($p<0.001$) and Potassium ($p<0.01$). Although, quercetin co-treated with water-soluble fraction ameliorates the adverse effect of water-soluble fraction on the levels of malondialdehyde, superoxide dismutase, Zinc and Potassium; it could not ameliorate the effects on serum testosterone and IL-1beta. The study suggests that water-soluble induces testicular toxicity through increase in lipid peroxidation and testicular IL-1β levels which could have resulted in low testosterone levels.

Keywords: *Serum Testosterone, Crude oil, Oxidative stress, Inflammation, Testicular Toxicity*

INTRODUCTION

Crude oil, refined petroleum products, and polycyclic aromatic hydrocarbons are ubiquitous in various environmental compartments. Its exploration and transportation have generated a lot of environmental concerns, especially in developing nations (Raji and Hart, 2012). The devastating consequences of the crude oil spills in the Niger Delta of Nigeria pose public health hazards to both aerial and terrestrial environments (Raji and Hart, 2012). Crude oil has been shown to have adverse biological effects that are attributed to the dissolved low molecular

weight hydrocarbons and metallic ions which are the major components of the water-soluble fraction of crude oil (Rodriques *et al*., 2010). According to Gbotolorun *et al.* (2021), the fraction of Nigerian Bonny light crude oil soluble in water is relatively small to the total mass of crude oil; but it is this fraction that is a major determinant of the crude oil-induced toxicity.

There is increasing evidence indicating that crude oil causes cellular injuries by induction of oxidative stress through the formation of reactive oxygen species (ROS) and inhibiting the efficiency of the antioxidant defence system (Adesanya *et al*., 2009, Farombi *et al*., 2010; Ebokaiwe and Farombi, 2015). Ingestion of Nigerian Bonny light crude oil-contaminated feed has been attributed to environmental - toxin-induced oxidative damage to the antioxidant systems in the testes (Adesanya *et al*. 2009, Farombi *et al.* 2010). Furthermore, Ebokaiwe and Farombi (2015), reported that the ingestion of Bonny light crude oil significantly decreased serum testosterone in male Wistar rats after 6 weeks of administration. Moreover, an increase in oxidative stress has also been shown to increase the production of inflammatory cytokines like Interleukins and Tumour Necrotic factors. Additionally, oxidative stress-induced cytokine production is likely to further increase oxidative stress levels (Elmarakby and Sullivan, 2012). However, Ebokaiwe and Farombi (2015) have reported the protective role of quercetin on Nigerian Bonny Light crude oil-induced oxidative stress in rat testes.

Quercetin (3, 3', 5, 7-pentahydroxyflavone) is one of the most abundant bioflavonoids in the human diet (Duarte *et al*., 2001). According to Egert *et al.* (2011), quercetin is widely distributed in plant-food including onions (*Allium cepa*), unpeeled apples, berries, citrus fruits, tea (*Camellia sinensis*) and red wine. Several studies have shown that it prevents oxidant injury and cell death via several mechanisms, such as scavenging oxygen radicals, protecting against lipid peroxidation and chelating metal ions (Bischoff, 2008; Ebokaiwe and Farombi, 2015). Moreover, increasing evidence has revealed the protective effect of dietary quercetin against oxidative toxicity on rat liver, renal, testis and sperm (Morales *et al*., 2006; Liu *et al*., 2010; Oyeyemi *et al*., 2022). The protective effect of quercetin on neuronal and testicular toxicity induced by the ingestion of bonny light crude oil has been reported. However, this study seeks to investigate the protective effects of quercetin on the water-soluble fraction of Nigerian Bonny light crude oil-induced testicular toxicity in male Wistar rats.

MATERIALS AND METHODS

Chemicals and reagents

The quercetin used for this study was purchased from Sigma Chemical Corporation (St. Louis, MO, USA), while the Nigerian Bonny light crude oil (BLCO) were obtained from the Nigeria Pipeline Product Marketing Company Limited, Subsidiary of the Nigerian National Petroleum Cooperation, Port Harcourt refinery branch. The water-soluble fraction was prepared according to the method of Anderson *et al. (*1974) with slight modification as described by Ogali *et al.* (2007).

Experimental animal design

Twenty (20) healthy male Wistar rats weighing about 100-120g were obtained from the Department of Physiology, Enugu State University of Science and Technology, Nigeria, and were randomly selected into four major groups of five (5) animals each; Control (C), Water soluble fraction of BLCO (WSF), Quercetin (QE) and Water-soluble fraction + Quercetin (WSF+QE**).** The animals were acclimatized to standard laboratory conditions for two (2) weeks with feed and water supplied *ad libitum.* Animals were gavage daily with 1ml of distilled water (control), 1ml of 50% of WSF (Patrick-Iwuanyanwu *et al*., 2013) and quercetin at a dose of 10 mg/kg body weight (Ebokaiwe and Farombi, 2015) for six weeks respectively. The guide for the care and use of laboratory animals, by the Institution for Laboratory Animals Research (ILAR 2011) was strictly followed.

At the end of the six (6) weeks, all the animals were anaesthetized under ether anaesthesia. Blood samples were collected through a cardiac puncture, and the testes were harvested. The animals were euthanised by cervical dislocation and, the bodies were properly disposed of.

Hormone and Inflammatory Marker

The Enzyme-linked immunosorbent assay (ELISA) technique was used to determine serum testosterone and testicular Interleukin-1 beta (IL-1β using Calbiotech kits (El Cajon, California). The procedures for the estimation of testosterone and interleukin-1 beta were carried out according to the kits' manual.

Testicular oxidant and antioxidant activity

The testes were homogenised, and the supernatant obtained was used for the assays. Malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) activities were determined according to the studies of Kunle-Alabi *et al.* (2017) and Oyeyemi *et al* (2020).

Testicular sodium, potassium, zinc and copper

Testicular sodium (Na), potassium (K), zinc (Zn), and copper (Cu) levels were determined by atomic absorption (AA) flame spectrophotometry techniques. The absorbances

of Na, K, Zn and Cu were read at 589.0 nm, 766.5 nm, 213.9 nm, and 324.8 nm respectively (Demarini *et al*., 2017).

Statistical analysis

Data were expressed as mean ±S.E.M and analyzed using GraphPad Prism (Version 8.0.1). One–way Analysis of Variance (ANOVA) was used to determine the mean difference among the treatment groups and followed by Bonferroni's post hoc test. The level of significance was accepted at $P \leq 0.05$.

RESULTS

The effect of quercetin on the testicular weight of Wistar rats treated with the water-soluble fraction of crude oil is presented in Figure 1. The result shows no significant differences in the mean testicular weight across the different treatment groups.

The present study shows that serum testosterone significantly decreased in male Wistar rats treated with the water-soluble fraction of crude oil compared to control (P<0.001). Moreover, quercetin co-treated with the water-soluble fraction of crude oil could not ameliorate the effect of water-soluble crude oil on serum testosterone (Figure 2).
In Figure 3, the result indicates a significant increase in testicular Interleukin-1beta in the water-soluble fraction of the crude oil-treated group when compared to control (P<0.01). However, quercetin could not ameliorate the effect of the water-soluble fraction of crude oil on testicular interleukin-1beta in the co-treated group.

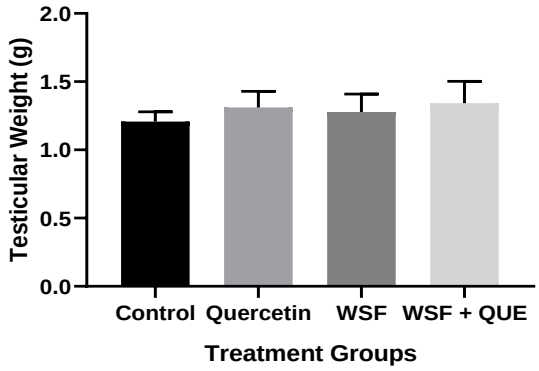

Figure 1. The Effect of Quercetin on Testicular and Weight of Wistar Rats Treated with Water Soluble Fraction of Crude Oil.

Data are presented in mean ± SEM; n=5

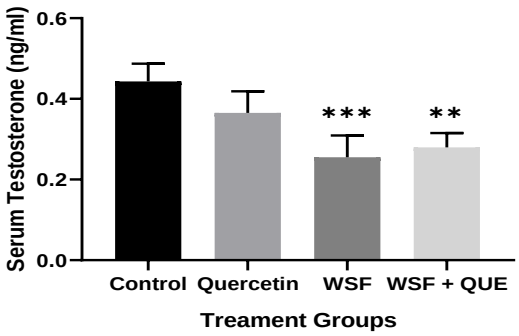

Figure 2. The Effect of Quercetin on Serum Testosterone Levels in Male Wistar Rats Treated with Water Soluble Fraction of Crude Oil.

Data are presented in mean ± SEM; n=5; ***p<0.001, **p<0.01, *P<0.05. *Statistically significant when compared to Control.

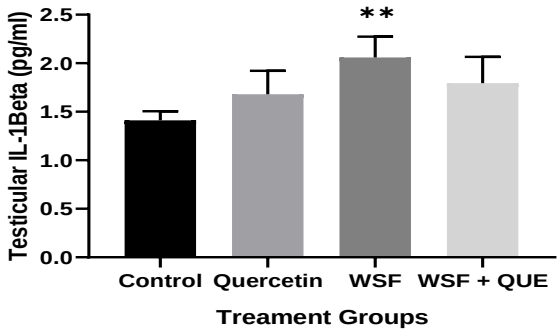

Figure 3. The Effect of Quercetin on Testicular Interleukin – 1β in Male Wistar Rats Treated with Water Soluble Fraction of Crude Oil.

Data are presented in mean ± SEM; n=5; ***p<0.001, **p<0.01, *P<0.05. *Statistically significant when compared to Control.

The result in Figure 4 shows that the testicular level of MDA increased significantly (P<0.001), and SOD activities were significantly reduced (P<0.01) in the water-soluble fraction of the crude oil-treated group when compared with the control group (Figure 4a, 4c). The co-administration of quercetin with the water-soluble fraction of crude oil significantly decreased (P<0.05) the testicular MDA level compared with the water-soluble fraction of the crude oil group (Figure 4a). There was a significant increase (P<0.05) in the testicular activities of SOD in the group co-treated with quercetin and the water-soluble fraction of crude oil compared to the water-soluble fraction of crude oil-treated group (Figure 4c). Interestingly, no significant difference in testicular catalase activity across the treatment groups was observed (Figure 4b).

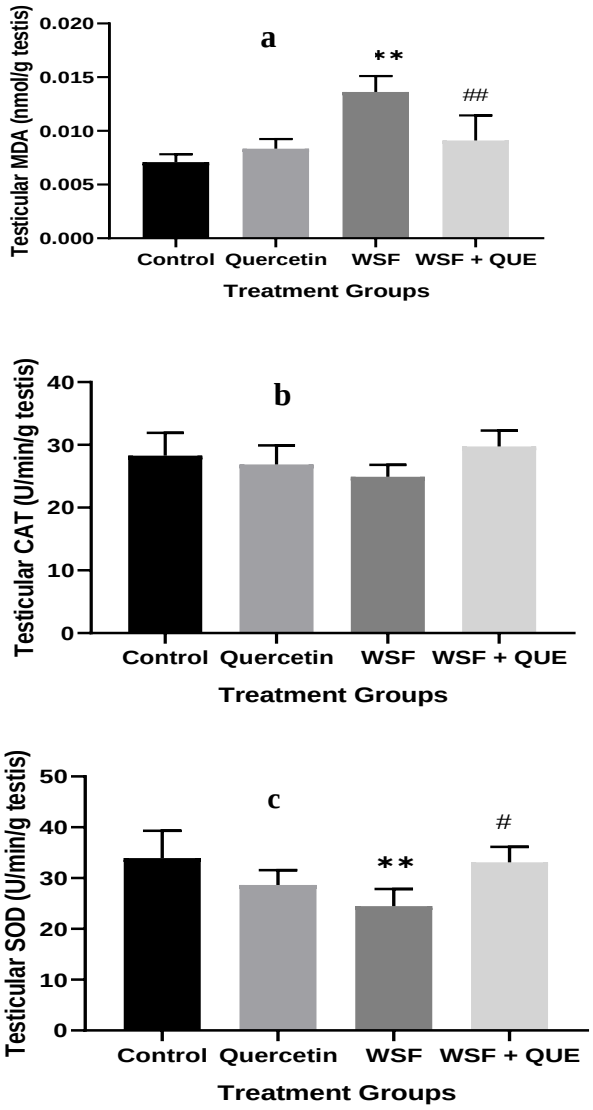

Figure 4. The Effect of Quercetin on Testicular Oxidant and Antioxidants in Male Wistar Rats Treated with Water Soluble Fraction of Crude Oil.

Data are presented in mean ± SEM; n=5; ***p<0.001, **p<0.01, *P<0.05, #p<0.05, ##p<0.01. *Statistically significant when compared to Control. #statistically significant when compared to WSF.

The effects of quercetin on selected testicular elements in male Wistar rats with the water-soluble fraction of crude oil are presented in Table 1. The result shows that testicular potassium and zinc levels significantly decreased in the water-soluble fraction of crude oil-treated group compared to the control group (P<0.01). Moreover, quercetin significantly increases testicular levels of potassium and zinc in the co-administration of quercetin and the water-soluble fraction of the crude oil group when compared with the water-soluble fraction of crude oil-treated group (P<0.01). In addition, testicular levels of sodium and copper were not significantly different across the treatment groups.

Table 1. The Effect of Quercetin on Selected Testicular Elements in Male Wistar Rats Treated with Water Soluble Fraction of Crude Oil.

Treatment	Potassium (mmol/l)	Sodium (mmol/l)	Zinc (µmol/l)	Copper (µmol/l)
Control	11.97 ± 0.38	125.21 ± 1.65	10.94 ± 0.24	23.07 ± 1.53
Quercetin	11.42 ± 0.23	130.00 ± 1.23	11.14 ± 0.42	23.01 ± 0.69
WSF	9.91 ± 0.35**	127.90 ± 1.25	8.68 ± 0.26***	26.37 ± 2.21
WSF + QUE	11.90 ± 0.36##	128.42 ± 2.03	10.76 ± 0.29##	28.12 ± 1.93

Data are presented in mean ± SEM; n=5; ***p<0.001, **p<0.01, *P<0.05, #p<0.05, ##p<0.01. *Statistically significant when compared to Control. #statistically significant when compared to WSF.

DISCUSSION

The present study investigated the impact of crude oil on testicular functions and the protective effect of quercetin. In this study, the mean testicular weight and epididymal weight of Wistar rats had no significant differences across the different treatment groups when compared to the control group. This finding is not consistent with the study of Adeleke *et al.* (2018), who reported that exposure to crude oil caused a significant increase in body weight gain in experimental rats relative to control, suggesting the tendency of this toxicant to induce overweight or even obesity in mammals. However, Orisakwe *et al.* (2004) reported a decrease in Wistar rat testicular weight when exposed to crude oil. The weight of the male reproductive organ usually provides a useful reproductive risk assessment in experimental studies (Raji *et al.*, 2005).

Testosterone is the male gonadal hormone produced by the interstitial cells of the Leydig in the testis. The testicular steroidogenesis can be affected by many exogenous factors (chemicals, pollutants, e.t.c) acting in many different ways due to the complex physiological mechanism that regulates Leydig cell function (Cooke, 1998; Ebokaiwe and Farombi, 2014). The reduction in serum testosterone level in this study, is consistent with the study of Ebokaiwe and Farombi (2014), which reported a significant decrease in serum testosterone levels in rats administered with Bonny light crude oil. The decreased serum testosterone in the water-soluble fraction of crude oil-treated rats observed may likely be due to Leydig cell impairment caused by ROS generation (Chen *et al.*, 2008).

There was a significant increase in testicular MDA in rats treated with WSF compared to the control in this study. This finding is consistent with previous studies on Bonny light crude oil (Farombi *et al.*, 2010; Ebokaiwe and Farombi, 2014). The increase in testicular MDA indicates the

induction of lipid peroxidation in the testis, which might have contributed to the loss of testicular function due to oxidative damage (Oyovwi *et al.*, 2021). This finding implies that the water-soluble fraction of crude oil exerts testicular toxicity in Wistar rats. In addition, antioxidant enzymes SOD were adversely affected by WSF in testes. SOD plays a key role in the detoxification of superoxide radicals, thereby protecting the testis from damage induced by free radicals (Fridovich, 1997). The observed decrease in SOD activity suggests increased production of superoxide radicals due to exposure to WSF. In a related study, Azeez *et al.* (2013) reported a significant reduction in SOD activity on exposure to petroleum hydrocarbons. However, the administration of quercetin was effective in ameliorating testicular toxicity induced by lipid peroxidation in the group co-treated with WSF compared to control in this study. This finding is in line with the study of Ebokaiwe and Farombi, (2014), that have reported the antioxidant effects of quercetin on crude oil-induced testicular toxicity. Quercetin is efficacious in stabilizing lipid membranes and preventing lipid peroxidation through its mechanism of free radical scavenging (Cerezetti *et al.*, 2021). Surprisingly, our study could not show significant effects on the testicular activity of catalase across the different treated groups. This finding implies that WSF had no adverse effect on the catalase activity of the testes of Wistar rats.

Another possible pathway that may account for the reduced serum testosterone in the WSF treated group may be the inflammatory pathway. Several studies have documented that inflammatory cytokines can inhibit cAMP-induced steroidogenesis (Payne and Youngblood, 1995; Guzman *et al.*, 2010; Milosevic *et al.*, 2021). It has been shown that a single injection of IL-1β (an inflammatory cytokine) can reduce the effects of hCG stimulation on testosterone production (Turnbull and Rivier, 1997). The significant increase in testicular IL-1β in WSF treated group in this study may account for the low serum testosterone level recorded in the WSF-treated group when compared to the control group. This study is in line with the study of Ita *et al.*, (2016), that reported increase in inflammatory markers and reduced testosterone in crude oil-induced toxicity. Surprisingly, quercetin could not ameliorate the increased testicular inflammatory biomarkers in the QUE+WSF co-treated group compared to the WSF treated group. This finding could likely explain the significant reduction in serum testosterone levels in QUE +WSF co-treated group compared with the control despite the antioxidant effects of quercetin.

Finally, this study observed that WSF significantly reduced the testicular level of zinc and potassium. This finding is consistent with Oyeyemi *et al*. (2022), who reported a similar outcome. Zinc is an antioxidant element that is available in body tissues and fluids. It is essential for cell division, growth, immunity, and wound healing. The observed decrease in the testicular zinc levels may likely be due to WSF interrupting its binding site in the testes. However, the co-administration of WSF with quercetin increases testicular zinc and potassium levels, suggesting that quercetin could reduce testicular toxicity induced by WSF due to its antioxidant and chelating properties. Additionally, testicular levels of sodium and copper were not significantly different across the treatment groups.

CONCLUSION

The study suggests that WSF induces testicular toxicity through increase in lipid peroxidation and testicular IL-1β levels which could have resulted in the low testosterone level. Moreover, quercetin could only ameliorate the effects of WSF on testicular lipid peroxidation.

AUTHORS' CONTRIBUTIONS

Author OBO and EWU designed the study and participated in data acquisition and analysis. Author GEO, TFA, and PGO participated in data generation, computation and manuscript drafting. Author VE, OMO, and MLN contributed to the manuscript. All the authors approved the final version of the manuscript.

FUNDING STATEMENT

This research was supported by TETFUND. EWU received the funding from TETFUND. TETFUND had no role in the study design, data collection and analysis, or preparation of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

REFERENCES

Adeleke, G.E., Badmus, J.A., Adedosu, O.T., Abioye, D.H. and Arinde, O.O. (2018). Betulinic acid attenuates hepatic and testicular redox imbalance and DNA damage in male rats exposed to crude oil. *Journal of Pharmacognosy and Phytochemistry*; 7:316-322.

Adesanya, O. A., Shittu, L. A. J., Omonigbehin, E. A. and Tayo, A. O. (2009). Spermatotoxic impact of bonny light crude oil (BLCO) ingestion on adult male Swiss albino mice, *International Journal of Physical Sciences;* 4 (5),349-353

Anderson, J.W., Neff, J.M., Cox, B.A., Tatem, H.E. and Hightower, G.M. (1974). Characteristics of dispersions and water soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. *Marine Biology*; (7): 75-88

Azeez, O.M., Akhigbe, R.E. and Anigbogu, C.N. (2013). Oxidative status in rat kidney exposed to petroleum hydrocarbons. *Journal of Natural Science, Biology and Medicine*; 4:149.

Bischoff, S.C. (2008). Quercetin: potentials in the prevention and therapy of disease. *Current Opinion Clinical Nutrition Metabolic Care*; 11,733-740

Cerezetti, M. B., González, S. M., Ferraz, C. R., Verri, W. A., Rabelo, E. A., Seneda, M. M. and Morotti, F. (2021). Impact of the antioxidant quercetin on morphological integrity and follicular development in the in vitro culture of Bos indicus female ovarian fragments. *In Vitro Cellular & Developmental Biology-Animal*; *57*(9), 856-864.

Chen, H., Pechenino, A.S. and Liu, J., (2008). Effect of glutathione depletion on Leydig cell steroidogenesis in young and old brown Norway rats. *Endocrinology;* 149: 2612–2619.

Cooke, P.S., Buchanan, D.L., Lubahn, D.B. and Cunha, G.R. (1998). Mechanism of estrogen action: lessons from the estrogen receptor-α knockout mouse. *Biology of Reproduction*; 59(3):470-5.

DeMarini, D.M., Warren, S.H., Lavrich, K., Flen, A., Aurell, J., Mitchell, W., Greenwell, D., Preston, W., Schmid, J.E., Linak, W.P. and Hays, M.D. (2017). Mutagenicity and oxidative damage induced by an organic extract of the particulate emissions from a simulation of the deepwater horizon surface oil burns. *Environmental and Molecular Mutagenesis*; 58(3):162-71.

Duarte, J., PeÂrez-Palencia, R., Vargas, F., Ocete, M.A., PeÂrez-Vizcaino, F. and Zarzuelo, A. (2001). Antihypertensive effects of the flavonoid quercetin in spontaneously hypertensive rats. *British Journal of Pharmacology;* 133: 117-124

Ebokaiwe, A.P. and Farombi, E.O. (2015). Influence of vitamin E and quercetin on Nigerian Bonny Light crude oil-induced neuronal and testicular toxicity in Wistar rats. *Journal of Basic and Clinical Physiology and Pharmacology;* 26:223-231.

Egert, S., Wolffram, S., Schulze, B., Langguth, P., Hubbermann, E.M. and Schwarz, K. (2011). Enriched cereal bars are more effective in increasing plasma quercetin compared with quercetin from powder-filled hard capsules. *British Journal of Nutrition*; 107(4),539-546.

Elmarakby, A.A. and Sullivan, J.C. (2012). Relationship between oxidative stress and inflammatory cytokines in diabetic nephropathy. *Cardiovascular Therapeutics*; 30(1):49-59.

Farombi, E.O., Adedara, I.A., Ebokaiwe, A.P., Teberen, R. and Ehwerhemuepha, T. (2010). Nigerian Bonny Light Crude Oil disrupts dntioxidant systems in testes and sperm of rats; *Journal of Archives of Environmental Contamination and Toxicology*, 59:166-174

Fridovich, I. (1997). Superoxide anion radical (O· 2), superoxide dismutases, and related matters. *Journal of Biological Chemistry*; 272(30),18515-185157.

Gbotolorun, S. C., Ezeife, C. C. and Ogunlade, B. (2021). Prenatal exposure of bonny light crude oil induces embryotoxicity, impaired cognitive functions and cortico-hippocampal neurodegeneration on fetal outcomes of pregnant sprague-dawley rats. *Drug and Chemical Toxicology;* 45(5),1975-1985

Guzman, C., Hernández-Bello, R. and Morales-Montor, J. (2010). Regulation of steroidogenesis in reproductive, adrenal and neural tissues by cytokines. *Open Neuroendocrinology Journal;* 3161-169

Ita, S.O., Akpanyung, E.O., Robert, A.S., Aluko, E.O., Eseabasi, F.O. and Sunday-Essien, M.U. (2016). Evaluation of some inflammatory biomarkers in male Albino wistar rats following ingestion of crude oil and the role of bee honey, vitamins C and E. *Modern Research in Inflammation*; 5(3),55-62.

Kunle-Alabi, O.T., Akindele, O.O., Odoh, M.I., Oghenetega, B.O. and Raji, Y. (2017). Comparative effects of coconut water and N-Acetyl cysteine on the hypothalamo-pituitary-gonadal axis of male rats. *Songklanakarin Journal of Science and Technology*; 39(6), 759-764

Liu, C.M., Zheng, Y.L., Lu, J., Zhang, Z.F., Fan, S.H. and Wu, D.M. (2010). Quercetin protects rat liver against lead-induced oxidative stress and apoptosis. *Environmental Toxicology Pharmacology*; 29:158–166.

Milosevic, A., Bjelobaba, I., Bozic, I.D., Lavrnja, I., Savi, D., Tesovic, K. and Janjic, M.M. (2021). Testicular steroidogenesis is suppressed during experimental autoimmune encephalomyelitis in rats. *Scientific Reports*; 11:1-12.

Morales, A.I., Vicente-Sánchez, C., Sandoval, J.M., Egido, J., Mayoral, P. and Arévalo, M.A. (2006). Protective effect of quercetin on experimental chronic cadmium nephrotoxicity in rats is based on its antioxidant properties. *Food Chemical Toxicology;* 44:2092–100.

Ogali, R.E., Osuji, L.C. and Ayodele, O. (2007). Acute Toxicity of the Water- Soluble Fraction of Spent Lubricating Oil on the African Catfish Clarias gariepinus. *Chemistry & Biodiversity; 4: 2755–2765*

Orisakwe, O.E., Akumka, D.D., Njan, A.A. and Afonne, O.J. (2004). Testicular toxicity of Nigerian bonny light crude oil in male albino rats. *Reproductive Toxicology*, 18:439-442.

Oyeyemi, W.A., Akinola, A.O., Daramola, O.O.O., Aikpitanyi, I., Durotoluwa, O.T., Alele, P.G.O. and Okoro, T.D. (2022). Vitamin E and quercetin attenuated

the reproductive toxicity mediated by lead acetate in male Wistar. *Bulletin of the National Research Centre*; 46:1-10.

Oyeyemi, W.A., Daramola, O.O.O., Akinola. A.O., Idris, A.O. and Aikpitanyi, I. (2020). Hepatic and reproductive toxicity of sub-chronic exposure to dichlorvos and lead acetate on male Wistar rats. *Asian Pacific Journal of Reproduction*; *9*:283-290.

Oyovwi, M.O., Nwangwa, E.K., Ben-Azu, B., Rotu, R.A., Edesiri, T.P., Emojevwe, V., Igweh, J.C. and Uruaka, I.C. (2021). Prevention and reversal of chlorpromazine induced testicular dysfunction in rats by synergistic testicle-active flavonoids, taurine and coenzyme-10. *Reproductive Toxicology*; 101:50-62

Patrick-Iwuanyanwu, K.C., Okon, E.A. and Nkpaa, K.W. (2013). Hepatotoxicological evaluation of water-soluble fraction (WSF) of bonny light crude oil (BLCO) in Wistar albino rats. *Biokemistri;* 25: 17-22.

Payne, A.H. and Youngblood, G.L. (1995). Regulation of expression of steroidogenic enzymes in Leydig cells. *Biology Reproduction*; 52,217–225.

Raji, Y. and Hart, V.O. (2012). Influence of prolonged exposure to Nigerian Bonny light crude oil on fertility indices in rats. *Nigerian Journal of Physiological Sciences;* 27:055 – *063*

Raji, Y., Akinsomisoye, O. S. and Salman, T. M. (2005). Antispermatogenic activity of Morinda lucida extract in male rats. *Asian Journal of Andrology*, *7*(4), 405-410.

Rodrigues, R.V., Miranda-Filho, K.C., Gusmão, E.P., Moreira, C.B., Romano, L.A., and Sampaio, L.A. (2010). Deleterious effects of water-soluble fraction of petroleum, diesel and gasoline on marine pejerrey *Odontesthes argentinensis* larvae. *Science of the Total Environment*; 408: 2054–2059.

Turnbull, A.V. and Rivier, C. (1997). Inhibition of gonadotropin-induced testosterone secretion by the intracerebroventricular injection of interleukin-1 beta in the male rat. *Endocrinology;* 138:1008–1013.