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Protective Effects of Vitamins E and C Against Crude Oil Induced Reproductive Toxicity in Rats

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Abstract

This study determined some female serum sex hormones, litter size, birth weight and tail lengths of offspring's delivered by rats orally exposed to crude oil and concomitantly administered vitamins E and C, respectively; using one hundred and two (102) albino Wistar rats (24 males and 78 females), weighing 160 to l80g. The rats were respectively distributed into six groups, with seventeen rats (four males and thirteen females) in each group. Groups 1, 2, 3, 4, 5 and 6 animals were given distilled water, 600 IU/kg of vitamin C only, 400 IU/kg of vitamin E only, 60mg/kg bow of crude oil only, 60mg/kg bow of crude oil with concomitant administration of 400 IU/kg of vitamin E, and 60mg/kg bow of crude oil with concomitant administration of 600 IU/kg of vitamin C orally for 30 days, respectively. After 30 days, five female rats from each group were sacrificed and blood serum collected for sex hormonal assay. The remaining rats were mated (one male to two females) until pregnancy was observed, after which they were withdrawn from experimental treatments. On delivery, the litter size for each group was recorded, total body weight and tail length of each litter were measured at birth and at weekly intervals for six weeks. The results of this study showed that crude oil produced a significant ($p<0.05$) decrease in serum estradiol and progesterone levels in female rats, compared to control; and that vitamins C and E attenuated this effect by producing a significant increase in the hormonal levels of rats exposed to crude oil. Also, a significant ($p<0.05$) decrease in litter size was recorded for rats given crude oil only, compared to the control. The mean total body weight of the litters delivered by rats treated with crude oil, at birth was insignificantly lower, compared to those in control. However, the weekly mean total body weight of the litters from the group treated with crude oil, for six weeks, was significantly ($p<0.05$) lower, compared to the litters from rats in the control group. Also, the mean tail length of the litters delivered by rats treated with crude oil, at birth and weekly for six weeks were insignificantly ($p>0.05$) lower, compared respectively, to the control group at corresponding intervals. The results also indicated that administration of vitamins C and E, respectively, to rats exposed to crude oil produced a significant reversal of the crude oil-induced decrease in litter size, birth weight and litter tail lengths in rats. It may therefore be concluded that vitamins C and E may be used to ameliorate crude oil-induced reproductive toxicity (decreased serum estradiol and progesterone, litter size, birth weights and tail length) in rats.

Keywords: Crude Oil; Litter Size; Litter Weight, Litter Tail Length, Serum Sex Hormones.

Introduction

The health risk associated with exposure to pollutants in the environment has been a major concern in recent times. Petroleum (or crude oil) and petroleum products are among the common sources of environmental pollutants in most oil producing societies, including Nigeria. Pollution from petroleum and petroleum products may arise from the evaporation of the volatile constituents of the oil in the course of production or processing, transportation, storage and utilization of the products. This may occur accidentally or operationally wherever crude oil is produced

or its products processed, transported, stored and used [1]. Crude oil and most of its fractionated products are known to consist of a complex mixture of several hydrocarbons and metals. Inadvertent exposure to crude oil or its complex chemical constituents have been observed to cause some toxic effects in humans, livestock and other animal species [2]. However, it has been reported that the toxic effects associated with crude oil-induced pollutants depends on such factors as the nature and type of the crude oil, the type of environment and degree or level of environmental contamination, as well as the level or degree of sensitivity and vulnerability of the organisms [3]. The toxicity effects of crude oil, and its products, are likely to be related to the cellular damage through protein oxidation and lipid peroxidation caused by the reactive oxygen and other species generated from the metabolism of the hydrocarbon contents of these products in the body [4].

Among other toxicity effects reported be associated with crude oil include reduction in body weight and the weights of such vital organs as the testis, liver, kidney, heart and pancreas in rabbits [5,6]. reduction in red blood cell and hematocrit value in Guinea pigs [7] alterations in hematological characteristics and performance in goats [6]. alterations in the hormonal system, which may affect the reproduction process in rats [8]. Also, polyromantic hydrocarbons are known to be among the components of crude oil with carcinogenic, mutagenic, and other negative impact on the marine environment [9-11]. Exposure of fish to marine environment polluted with high levels of these hydrocarbons may lead to immunosuppression, or death of the fish [12-15]. Generally, several toxicological hazards and/or properties have been reported to be associated with the exposure to different hydrocarbon solvents with complex and variable compositions, including alkanes (normal paraffin's, isoparaffins, and cycloparaffins) and aromatics [16].

Provision of protection against the various toxicity effects associated with exposure to chemical pollutant is a subject of major concern to the environmental and biochemical toxicologists in the recent times. Different antioxidants are known to play a role in preventing the tissues against the toxicity effects associated with the interaction of reactive species generated from the metabolism of toxicants with the body tissues. Antioxidant vitamins are particularly known to play an important role in ameliorating the toxicity effects of reactive species generated by chemical agents in the biological systems. Hence, the use of antioxidant vitamins to ameliorate the chemical agents-induced toxicity has attracted the attention of biochemical and toxicological researchers in last few decades [17,14,18-21]. Our previous studies reported that vitamins A, C and E play a role in ameliorating the adverse effect associated with exposure to gasoline vapor on the female serum sex hormonal levels in rats [22]. Also, protective effect of vitamins C and vitamin E against gasoline vapor-induced liver injury have been demonstrated [23]. This present study assessed the litter size (number of litters), tail length, and birth weight of offspring's delivered by rats orally exposed to crude oil and concomitantly administered, respectively, vitamins E and C.

Materials and Methods

Crude Oil

Bonny Light Crude oil was collected from the NNPC/ TOTAL/SHELL Joint venture in Port Harcourt through procedural authorization from the Department of Petroleum Resources Zonal Office, Port Harcourt, Nigeria

Animal handling and treatment

One hundred and two (102) albino Wistar rats (twenty-four males and seventy-eight females), with the weights ranging between 160 to l80g were used in this research work. The animals, obtained from Biochemistry and Pharmacology Departments of University of Calabar, Nigeria, were maintained with normal feeds, and allowed one week to acclimatize to the experimental conditions of twelve hours' light cycle and ambient temperature, before the research experiment commenced.

The animals were fed with pellet sized feed (Agro Feeds Ltd., Calabar) and allowed access to tap water ad libitum. The male and female rats were respectively distributed into four groups, with seventeen rats (four males and thirteen females) in each group. Preliminary acute toxicity study in mice, gave LD_{50} of 180mg/kg b. w t of crude oil (solubilized in Grand pure soya oil). Hence 60mg/kg b. w t of crude petroleum (approximately 60% of the LD_{50}) was used in this study. The animals' group distribution and treatment are as presented in Table 1.

Group	Treatment	Number of rats	
		Males	Females
1	Distilled water (Control)	4	13
2	600mg/kg of b.wt. of Vitamin C	4	13
3	400mg/kg of b.wt. of Vitamin E	4	13
4	60mg/kg body weight of Crude oil	4	13
5	60mg/kg b.wt. of Crude oil + 600mg/kg of b.wt. of Vitamin C	4	13
6	60mg/kg b.wt. of Crude oil + 400mg/kg of b.wt. of Vitamin E	4	13

Table 1: Distribution and treatment of experimental animals in the respective experimental groups.

The tablets of vitamin C, used in this study, were obtained from Emzor Pharmaceutical Industries, Lagos, Nigeria, while capsules of vitamin E were obtained from Shalina Laboratories, Pvt, Mumbai, India. Vitamin E was solubilized in vegetable oil, while vitamin C was solubilized in distilled water, as vehicles for oral administration. Prophylactic concentrations/dosages of the vitamins (i.e. 400 IU/kg for vitamin E, and 600 mg/kg for vitamin C), were administered to the rats, based on their total body weight [22]. Exposure to crude oil, with concomitant administration of the vitamins in solution, were done by oral garaging using intragastric syringe, as earlier described by [22]. The animals were orally exposed to crude oil and concomitantly treated, respectively with vitamins C and E daily for 30 days. After 30 days, five female rats from each group were sacrificed and blood serum collected for sex hormonal assay. The serum sex hormones (estradiol and progesterone) were analyzed using enzymes immunoassay methods. The respective immunoassay reagent kits were obtained from Diagnostic Automation Inc., 23961 Craftsman Road, Suite E/E, Calabasas, CA 91302. Microplate reader (Dialab Instruments Ltd.) was used in taking the absorbance. Calculations of the concentrations of hormones were made according to the method given in the kits handbook. The remaining rats were mated (one male to two females) until pregnancy was observed. Once pregnancy was detected, they were withdrawn from exposure and treatment until they delivered; and on delivery, the litter size (number of litters) for each group were recorded. Also, the total body weight and tail length of each litter (offspring) were taken at weekly interval for six weeks using standard animal weighing balance and metric meter rule, respectively. The study was carried out in accordance with the guidelines of the Institutional Animal Ethics Committee, following the NRC (1995) "Guide for the Care and Use of Laboratory Animals".

Results

The results of the effects vitamins E and C on serum female sex hormones, litter size and growth indices of the first generation litters of rats exposed to crude oil are shown in Figures 1 to 7. The results of the study on the effect of vitamins C and E on serum sex hormonal (progesterone and estradiol) profile of both sexes of rats exposed to crude petroleum are presented in Figures 1 and 2. The results showed that exposure to crude oil produced a significant decrease ($p<0.05$) in progesterone and estradiol levels in females (1.76 ± 0.16 and 2.72 ± 0.33mIU/l respectively), compared respectively with progesterone and estradiol levels for female (3.42 ± 0.57 and 3.88 ± 0.51 mIU/l, respectively) rats in the control groups. These results suggested that exposure to crude oil reduced sex hormonal level in female rats, an indication of endocrine disruption which may predisposes the organisms to reproductive dysfunctions. It was also observed that the progesterone and estradiol levels recorded for female rats exposed to crude oil and treated with vitamins C (3.10 ± 0.42 and 3.34 ± 0.50mmol/l respectively) and E (3.36 ± 0.48 and 3.40 ± 0.53mmol/l, respectively), were significantly ($p<0.05$) higher, compared respectively with the levels recorded for rats exposed to crude oil only. However, the serum sex hormonal profile recorded for the female rats exposed to crude oil, and administered vitamins C and E, were not significantly ($p>0.05$) different from the respective hormonal profile recorded for the control rats. This suggests that vitamins C and E are potent in providing protective measures against crude oil induced endocrine disruption, and attendant reproductive toxicity in both sexes of rats.

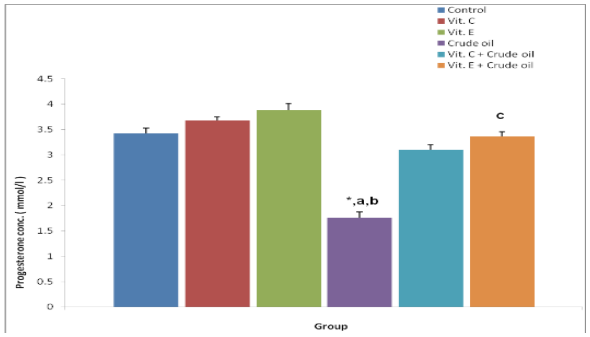

Figure 1: Comparison of progesterone concentrations between different experimental groups of female rats. Values are expressed as "mean ± SEM", n = 4; * $P<0.05$ compared with control group; a = $P<0.05$ compared with vit C group; b = $P<0.05$ compared with vit E group; c = $P<0.05$ compared with crude oil group.

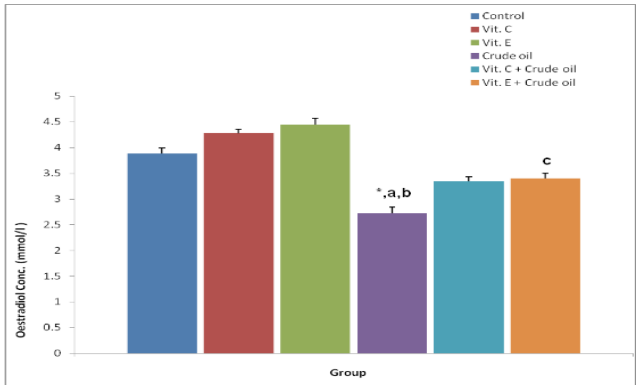

Figure 2: Comparison of Oestradiol concentrations between different experimental groups of female rats. Values are expressed as "mean ± SEM", n = 4; * $P<0.05$ compared with control group; a = $P<0.05$ compared with vit C group; b= $P<0.05$ compared with vit E group; c = $P<0.05$ compared with crude oil group.

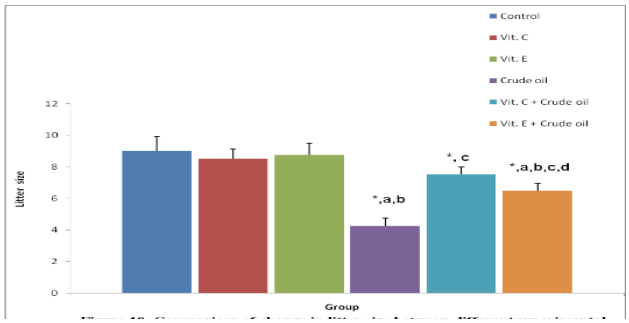

Figure 3: Comparison of change in litter size between the experimental groups of rats. Values are expressed as "mean ±SEM", n = 4; * P<0.05 compared with control group; a = P<0.05 compared with vit C group; b = P<0.05 compared with vit E group; c = P<0.05 compared with crude oil group; d = P<0.05 compared with Vit. C + crude oil group.

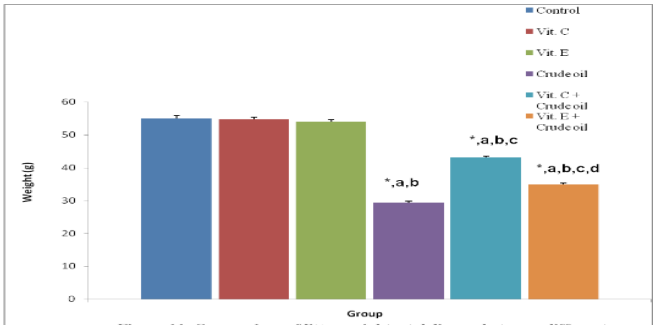

Figure 4: Comparison of litter weight, at delivery, between the experimental groups of ratsValues are expressed as "mean ±SEM", n = 4; * P<0.05 compared with control; a = P<0.05 compared with vit C group; b = P<0.05 compared with vit E group; c = P<0.05 compared with crude oil group; d = P<0.05 compared with Vit. C + Crude oil group.

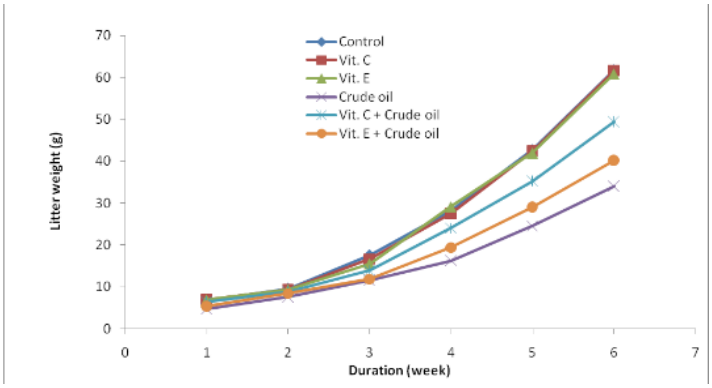

Figure 5: Changes in litter weight, within the first six weeks from delivery, between the experimental groups of rats. Values are expressed as "mean ±SEM", n= 7.

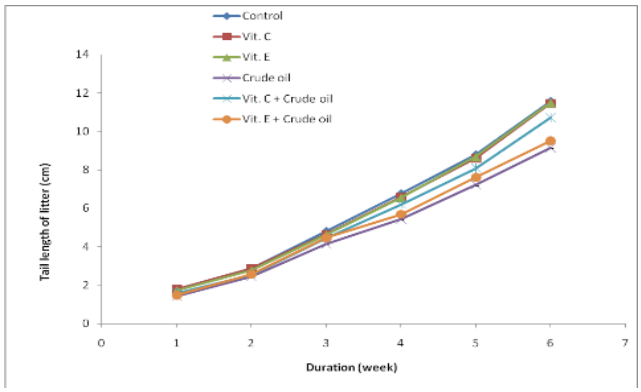

Figure 6: Weekly changes in tail length of litters between the experimental groups of rats. Values are expressed as "mean ±SEM", n = 7.

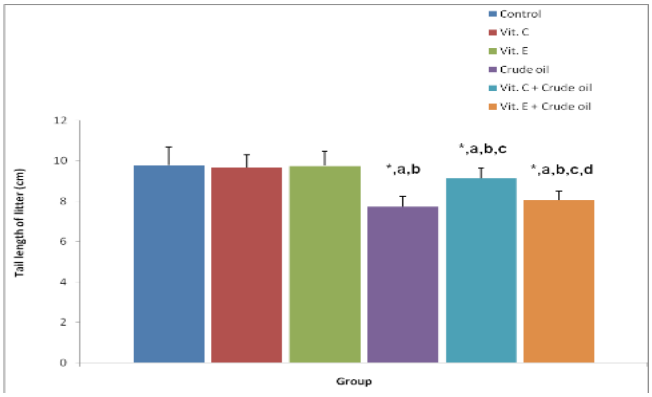

Figure 7: Average litter tail length between the experimental groups of rats after six weeks. Values are expressed as "mean ±SEM", n = 7; * P<0.05 compared with control group; a = P<0.05 compared with vit C group; b = P<0.05 compared with vit E group; c = P<0.05 compared with crude oil group; d = P<0.05 compared with Vit. C + Crude oil group.

The litter size (i.e., number of litters) delivered by rats exposed to crude petroleum and treated with vitamins E and C is presented in Figure 3. The results showed that litter size delivered by rats exposed to crude oil (4.25 ± 0.63) was significantly (p<0.05) lower, compared to the litter size delivered by rats in the control group (9.00 ± 0.41). The low litter size recorded for rats exposed to crude petroleum suggested that exposure to crude petroleum predisposed the animals to reproductive dysfunctions. However, the litter size delivered by rats exposed to crude oil and treated with vitamins C (7.50 ± 0.29) and E (6.50 ± 0.65), though significantly (p<0.05) lower than the litter size for the control group, was observed to be significantly (p<0.05) higher, compared to the litter size of rats exposed to crude petroleum only.

The growth indices (Total Body Weight and Tail Length) of the first generation litters of rats exposed to crude oil and treated with vitamins E and C, at birth and weekly changes for five weeks are shown in Figures 4 to 7. The results showed that the total body weights recorded for the litters delivered by rats in the control group, at birth and five subsequent weeks (6.86±0.12, 9.61±0.24, 17.60±0.32, 28.17±0.86, 42.71±0.62 and 61.79±1.02g, respectively), were significantly ($p<0.05$) higher, compared respectively with the total body weights recorded for the litters delivered by rats orally exposed to crude oil only (4.76±0.04, 7.66±0.07, 11.59±0.16, 16.33±0.83, 24.57±0.27 and 34.07±0.51g, respectively). The results showed that exposure to crude petroleum induced low birth weight in litters delivered by the exposed pregnant rats. However, the total body weights recorded for the litters delivered by rats exposed to crude petroleum and treated, respectively, with vitamins C (6.40±0.08, 8.83±0.13, 13.87±0.11, 24.03±0.10, 35.33±0.18 and 49.50±0.46g, respectively), and E (5.34±0.02, 8.40±0.05, 11.79±0.06, 19.37±0.36, 28.97±0.30 and 40.19±0.44g, respectively) were significantly ($p<0.05$) higher, compared with the litters delivered by rats exposed to crude petroleum only, though significantly lower, compared respectively with the litters delivered by rats in the control group. This indicated that administration of vitamins C and E can ameliorate the low birth weights condition associated with exposure to crude oil in rat model (Figures 4 and 5).

The results of this study also showed that the tail lengths recorded for the litters delivered by rats in the control group, at birth and five subsequent weeks (1.77±0.02, 2.86±0.04, 4.80±0.02, 6.76±0.03, 8.79±0.05 and 11.56±0.09cm, respectively), were significantly ($p<0.05$) higher, compared respectively with the tail lengths recorded for the litters delivered by rats orally exposed to crude petroleum only (1.41±0.01, 2.44±0.02, 4.13±0.02, 5.43±0.05, 7.20±0.05 and 9.14±0.02cm, respectively). However, the tail lengths recorded for the litters delivered by rats exposed to crude petroleum and treated respectively with vitamins C (1.59±0.01, 2.54±0.02, 4.46±0.05, 6.20±0.02, 8.09±0.03 and 10.73±0.08cm, respectively) and E (1.47±0.02, 2.56±0.02, 4.47±0.06, 5.69±0.03, 7.60±0.04 and 9.51±0.03cm, respectively) were insignificantly ($p>0.05$) higher, compared with the litters delivered by rats exposed to crude petroleum only, though insignificantly lower, compared respectively with the litters delivered by rats in the control group. This indicated that vitamins C and E can provide protection against any likely adverse effect of crude petroleum on the tail lengths and growth in rat model (Figures 6 and 7).

Discussion

The results of this study showed that exposure to crude oil produced low fecundity (Significant Reduction in Litter Size), as well as significant decrease in progesterone and estradiol levels in female rats. Also, low birth weights were recorded as one of the negative effects associated with exposure to crude oil. The low female sex hormones (progesterone and estradiol) levels recorded in this study for rats exposed to crude oil agreed with the results of the earlier studies reported by [24,25,22] for rats exposed to gasoline vapors. According to the authors, exposure to gasoline vapors significantly reduced sex hormone levels in rats. The low fecundity and reduction in sex hormonal levels reported in this study gave an indication of crude oil induced endocrine disruption which might have disposed the animals to reproductive dysfunctions. Reduced birth weights and premature birth have been reported for the offspring's delivered by pregnant humans exposed to cadmium [26]. Also, reduced fertility and low birth weights, as indicators of reproductive toxicity, have been reported in human beings that were occupationally exposed to such heavy metals as Cd, Pd and Hg; and petrochemical solvents [27-29]. Particularly, exposures to Cd during human pregnancy have been reported to cause reduced birth weights and premature birth [26]. The low fecundity and low birth weights of the litters reported for rats exposed to crude oil in this study supported the fact that sustained alterations in hormonal homeostasis can result in adverse effects. It also shows that alterations in hormonal homeostasis during development may have a serious detrimental effect on the developing embryo since the developing organism is known to be very sensitive to hormonal perturbations [30-34].

Birth weights and tail lengths (in experimental animals such as rats) have been reported to play a remarkable role in assessing chemical pollutants induced developmental toxicity [35]. In this study, remarkable low birth weights and tail lengths were recorded for litters delivered from the mating of male and female rats exposed to crude oil. In agreement with the result of this study, a significantly decreased body weight has been reported for the pups from the dams exposed to JP-8 jet fuel [36,37]. Other authors also reported similar observations in rats fed with crude oil contaminated feed [38]. According to the report of these authors, a significantly low body weights were recorded for litters produced by pregnant rats fed on crude oil contaminated feed, and impregnated by male adult rats also fed with the contaminated feed. Relatively shorter tail lengths were recorded for litters produced from the mating of both sexes of rats exposed to crude oil. Decrease in birth weights and tail lengths are among the physical manifestations of fetal developmental toxicity. Although the specific mechanism(s) of fetal developmental toxicity is (are) not very clear, [39] reported that prenatal exposures to both Methyl-Mercury and Polychlorinated Biphenyls (PCBs) are related to impaired neurodevelopment in infants. Methyl-Mercury and PCBs are industrial contaminants, belonging to the persistent organic pollutants. Reports from other authors have also demonstrated PCBs exposure to be inversely related to fetal growth [40,41]. Reciprocal relationship between blood PCB concentrations and birth weight has also been reported by [29]. [40] reported an inverse association between PCBs in

umbilical cord plasma and birth weight in one of their studies on Dutch infants born between 1990 and 1992. According to [42], the toxicity of the hydroxylase PCB metabolites might play important role in birth weight decrease, since they have been demonstrated to cross the placental barrier more efficiently than the parent compounds. Also, [43] in one of their studies reported estrogen levels in pregnancy to be linked to increased birth weight. Hence, the overall low serum estrogenic activity reported in this study to be associated with exposure to crude oil might be one of the mechanisms by which crude oil constituents, or their metabolites, would decrease birth weight.

It was also observed in this study that treatment of rats exposed to crude oil with vitamins C and E, respectively, produced a remarkable increase in progesterone and estradiol levels in the female rats. The results of this study are consistent with the results of the work carried out by [22] on rats exposed to gasoline vapor and treated separately with vitamins A, C and E. The authors reported that vitamins A, C and E are effective in preventing the adverse effects associated with gasoline toxicity on serum sex hormonal levels in female rats. It is generally known that antioxidants can scavenge or suppress the formation of ROS, thereby preventing the expression of ROS-induced oxidative stress on the animal tissues. Particularly, vitamin C and E have been reported to be potent antioxidant providing protection against deleterious effect of free radicals in the body tissues [44,45,20]. According to [17], vitamin E has an effective ant oxidative activity against lipid peroxidation. In agreement with the result of this study, [46], [47], [19], [48] demonstrated that vitamin C is an important hydrophilic antioxidant in the biological tissues. Also, [49] showed vitamin E as one of the most potent lipid-soluble antioxidant in biological tissues. It was interesting to observe from the results of the study that the vitamins did not express any significant effect on the parameters of unexposed rats (i.e., normal rats administered vitamins C and E only, without exposure to petroleum). It is therefore likely from the results of this study that vitamins C and E counteracted the ability of the toxicants in crude oils to induce reproductive toxicity in rat's model. It can be concluded from the results of this study that vitamins E and C can provide protection against any likely adverse effect of crude oil on the birth weights, tail lengths and growth in rat model. From this observation, it is evident that vitamins C and E may be used to attenuate crude oil induced developmental toxicity in rat model.

References

1. Orisakwe OE, Akumka DD, Njan AA, Afonne OJ (2004) "Testicular toxicity of Nigerian bonny light crude oil in male albino rats". Reproductive Toxicology 18: 439-442.
2. Coppock RW, Mostrom MS, Khan AA, Semalulu SS (1995) "Toxicology of oil field pollutants in cattle" a review. Vet Hum Toxicol 37: 569-576.
3. Garrity SD, Levings SC (1990) "Effects of oil spill on the gastropods of a tropical inter-tidal reef flat" Mar Environ Res 30:119-153.
4. Altenburger R, Nendza M, Schüürmann G (2003) "Mixture toxicity and its modeling by quantitative structure-activity relationships. Environ Toxicol Chem 22: 1900-1915.
5. Ovuru SS, Ekweozor IKE (2004) Haematological changes associated with crude oil ingestion in experimental rabbits. African Journal of Biotechnology 3: 346-348.
6. Ngodigha FM (2009) Haematological characterstics and performance of West African Dwarf Goats fed crude oil contaminated forage. African Journal of Biotechnology 8: 699-702.
7. Owu DU, Udoete UB, Azah N, Eyong EU (2005) Effect of Bonny light crude oil on some haematological parameters of guinea pigs. Biokemistri 17: 165 -170.
8. Olawale O, INE Onwurah (2007) Preliminary investigation into the possible endocrime disrupting activity of Bonny light crude oil contaminated diet on wistar albinorats. Biokemistri 19: 23-28.
9. Halek F, Nabi G, Kavousi A (2008) Polycyclic aromatic hydrocarbons study and toxic equivalency factor (TEFs) in Tehran, Iran. Environ Monit Assess 143: 303-311.
10. Bado-Nilles A, Quentel C, Mazurais D, Zambonino-Infante JL, Auffret M, et al. (2011) *In vivo* effects of the soluble fraction of light cycle oil on immune functions in the European sea bass, *Dicentrarchus labrax* (Linn_e). Ecotoxicol Environ Saf 74: 1896-1904.
11. Seker S (2012) EROD and PAH detection as biomonitoring tool in Golden Horn-Istanbul, Turkey. Int. J. Water Resour Environ. Eng 4: 139-143.
12. Danion M, Le Floch S, Lamour F, Guyomarch J, Quentel C (2011) Bioconcentration and immunotoxicity of an experimental oil spill in European sea bass (*Dicentrarchus labrax* L.). Ecotoxicol Environ Saf 74: 2167-2174.
13. Hicken C, Linbo T, Baldwin D, Willis M, Myers M, et al. (2011) Sublethal exposure to crude oil during embryonic development alters cardiac morphology and reduces aerobic capacity in adult fish. PNAS 108: 7086-7090.
14. Whitehead CC, Keller T (2003) An update on ascorbic acid in poultry. World's Poultry Sc J 59: 161 -184.
15. Omar-Ali A, Hohn C, Allen PJ, Rodriguez J, Petrie-Hanson L (2015) Tissue PAH, blood cell and tissue changes following exposure to water accommodated fractions of crude oil in alligator gar, *Atractosteus spatula*. Marine Environmental Research 108: 33- 44.
16. Mckee RH, Adenuga MD, Carrillo JC (2015) Characterization of the toxicological hazards of hydrocarbon solvents. Critical Reviews in Toxicology 45: 273-365.
17. Singhal S, Gupta R, Goyle A (2001) Comparison of antioxidant efficacy of vitamin E, vitamin C, vitamin A and fruits in coronary heart diseases. A controlled trial. J Assoc Phys India 49: 327 -331.
18. Son EW, Mo SJ, Rhee DK, Pyo S (2004) Vitamin C blocks TNF-alpha-induced NFkappa B activation and ICAM-1 expression in human neuroblastoma cells. Arch Pharmacol Res 27: 1073-1079.
19. Ayo JO, Minka NS, Mamman M (2006) Excitability scores of goats administered ascorbic acid and transported during hot-dry conditions. J Vet Sc 7: 127-131.

20. Ambali S, Akanbi D, Igbokwe N, Shittu M, Kawu M, et al. (2007) Evaluation of subchronic chlorpyriphos poisoning on haematological and serum biochemical changes in mice and protective effect of vitamin C. The Journal of Toxicological Science 32: 111-120.

21. Uboh FE, Ekaidem IS, Ebong PE, Umoh IB (2009) Hepatoprotective effect of Vitamin A against Gasoline vapours toxicity in rats. Gastroenterol Res 2: 162-167.

22. Uboh F, Akpanabiatu M, Edet E, Ebong P (2011) Vitamins A, C and E ameliorate gasoline vapor induced toxicity effect on sex hormonal levels in female Wistar rats. Acta Endocrinologica Buc 7: 189 -198.

23. Uboh FE, Ebong PE, Akpan HD, Usoh IF (2012) Hepatoprotective effect of vitamins C and E against gasoline vapor-induced liver injury in male rats. Turkish Journal Biology 36: 217-223.

24. Uboh FE, Akpanabiatu MI, Ekaidem IS, Ebong PE, Umoh IB (2007) Effect of Inhalation Exposure To Gasoline Vapours On Sex Hormones Profile In Wistar Albino Rats. Acta Endocrinologica (Buc) 3: 23-30.

25. Uboh FE, Ebong PE, Udosen EO (2010) Protective effect of vitamin C against gasoline vapours-induced reproductive toxicity in male wistar albino rats. Acta Endocrinologica Buc 6: 305-314.

26. Henson MC, Chedrese PJ (2004) Endocrine disruption by cadmium, a common environmental toxicant with paradoxical effects on reproduction. Experimental Biology and Medicine 229: 383-392.

27. Gerhard I, Monga B, Waldbrenner A, Runnebaum B (1998) Heavy metals and fertility. Journal of Toxicological Environmental Health 54: 593-611.

28. Grazuleviciene R, Dulskiene V, Vencloviene J (1998) Formaldehyde exposure and low birth weight incidence. Journal of Occupational Health 40: 61-67.

29. Hertz-Picciotto I (2000) The evidence that lead increases the risk of spontaneous abortion. American Journal of Induced Medicine 38: 300-309.

30. Shen H, Ong C (2000) Detection of oxidative DNA damage in human sperm and its association with sperm function and male infertility. Free Radical Biological Medicine 28: 529-536.

31. Vural P, Akgul C, Yildirim A, Canbaz M (2000) Antioxidant defence in recurrent abortion. Clinical Chim Acta; 295: 169-177.

32. Walsh SW, Vaughan JE, Wang Y, Roberts LJ (2000) Placental isoprostane is significantly increased in preeclampsia. FASEB Journal 14: 1289-1296.

33. Sikka SC (2001) Relative impact of oxidative stress on male reproductive function. Current Medical Chemistry 8: 851-862.

34. Uzumcu M. and Zachow R (2007) Developmental exposure to environmental endocrine disruptors: consequences within the ovary and on female reproductive function. Reproduction Toxicology 23: 337-352.

35. Hougaard K S, Jensen K A, Nordly P, Taxvig C, Vogel U, et al. (2008) Effects of prenatal exposure to diesel exhaust particles on postnatal development, behavior, genotoxicity and inflammation in mice. Particle and Fibre Toxicolog, 5.

36. Cooper JR, Mattie DR (1996) Developmental toxicity of JP-8 jet fuel in the rat. Journal of Applied Toxicology 16: 197-200.

37. NRC - National Research council (2001) Nutrient requirements of laboratory animals, (4th revised edn), National Academy Press. Washington, DC, pp.29-30.

38. Mitchell A, Bakshi K, Kimmel C, Buck G, Feuston M, et al. (2004) Evaluating chemical and other agent exposures for reproductive and developmental toxicity. Journal of Toxicological Environmental Health Association 67: 1159-1314.

39. Schettler T (2001) Toxic threats to neurologic development of children. Environmental Health Perspectives 109: 813-816.

40. Patandin S, Koopman-Esseboom C, de Ridder MA (1998) Effects of environmental exposure to polychlorinated biphenyls and dioxins on birth size and growth in Dutch children. Pediatric Research 44: 538-545.

41. Rylander L, Strömberg U, Dyremark E, Ostman C, Nilsson-Ehle P, et al. (1998) Polychlorinated biphenyls in blood plasma among Swedish female fish consumers in relation to low birth weight. American Journal of Epidemiology 147: 493-502.

42. Park J.S, Bergman A, Linderholm L (2008) Placental transfer of polychlorinated biphenyls, their hydroxylated metabolites and pentachlorophenol in pregnant women from eastern Slovakia. Chemosphere 70: 1676-1684.

43. NRC, (1995) National Research council: Nutrient requirements of laboratory animals fourth revised edition, National Academy Press, Washington, USA, 29-30.

44. Hanafy S, Soltan ME (2004) Effects of vitamin E pre-treatment on subacute toxicity of mixture of Co, Pb and Hg on nitrate-induced nephrotoxicity in rats. Environmental Toxicological Pharmacology 17: 159 - 167.

45. Ocak S, Gorur S, Hakverdi S, Celik S, Erdogan S (2007) Protective effect of caffeic acid phenethyl Esther, vitamin C, vitamin E and acetyl cysteine on vancomycin-induced nephrotoxicity in rats. Basic Clinical Pharmacological Toxicology 100: 328-333.

46. Chen K, Suh J, Carr AC, Morrow JD, Zeind J, et al. (2000) Vitamin C suppresses oxidative lipid damage in vivo, even in the presence of iron overload. American Journal of Physiology, Endocrinology Metabolism 279: 1406-1412.

47. Frei B (2004) Efficacy of dietary antioxidants to prevent oxidative damage and inhibit chronic disease. Journal of Nutrition 134: 3196-3198.

48. Akturk O, Demirin H, Sutcu R, Yilmaz N, Koylu H, et al. (2007) The effects of diazozin on lipid peroxidation and antioxidant enzymes in rats erythrocytes: role of vitamins E and C. TIH 23: 13-17.

49. Yarube IU, Emam Okasha MAH, Ayo JO, Olorunshola KV (2009) Antioxidant vitamins C and E alleviate the toxicity induced by chronic sodium nitrate administration on sperm count and serum testosterone level in Wistar rats. European Journal Scientific Research 25: 35-41.