Available online: www.notulaebiologicae.ro
Print ISSN 2067-3205; Electronic 2067-3264
Not Sci Biol, 2017, 9(3):332-337. DOI: 10.15835/nsb9310119
Original Article

Effects of Vitamin E Supplementation in Male Rats Affected by Crude Oil-Induced Reproductive Toxicity

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Abstract

Crude oil intoxication is a major threat among people and animals living around the crude oil producing regions of the world, hence the search for ameliorating agents. Forty-four male Wistar rats assigned to three groups were used to investigate the effects of vitamin E supplementation on crude oil-induced reprotoxicity (reproductive toxicity) in male rats. Group A represented the unexposed control, whereas groups B and C were exposed orally to 0.15 and 0.3 ml of crude oil respectively every other day, for 56 days. Both the low dose and high dose oral administration of crude oil caused a significant reduction in the serum testosterone level (STL) and cauda epididymal sperm reserve (CESR) of the exposed rats when compared to the control. Crude oil withdrawal and vitamin E supplementation significantly improved the cauda epididymal sperm reserve (CESR) in all the subgroups. The serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities of the control and low dose group were significantly lower than those of the high dose group. The high dose crude oil administration significantly decreased the mean serum total protein (STP) and sodium ions (Na^+) concentration. The mean serum total cholesterol (STC) value of the low dose group was significantly higher than those of the control and high dose group. However, crude oil withdrawal and vitamin E supplementation did not significantly alter the mean serum total protein (STP) and mean serum total cholesterol (STC) values in all the subgroups. Vitamin E supplementation following low dose crude oil withdrawal enhanced the mean serum Chloride ions (Cl^-) concentration. The present findings revealed that Nigerian Qua Iboe Brent crude oil induced serious reprotoxic effects in male rats which vitamin E administration within 28 days did not completely reverse.

Keywords: vitamin E, crude oil, testosterone level, sperm reserves, serum biochemistry

Introduction

Environmental pollution with petroleum products is a major feature in crude oil producing areas and can pose a serious health threat (Chang *et al.*, 2014). Aquatic and terrestrial animals, including humans living in such areas are particularly vulnerable to the hazardous effects emanating from crude oil spill pollutants (Burger, 1993). Thus inhabitants of oil-producing areas and workers in oil companies are at great risk of the pathologic effects of crude oil toxicants on diverse organs (Igwebuike *et al.*, 2004). Crude oil has the potential to elicit both acute lethal and chronic sub-lethal toxicities, depending on the means of exposure, dosage and animal species involved (Orisakwe *et al.*, 2004). It has been proven that crude oil has the capacity to induce toxic effects in some animal organs, like the lungs and liver (Kim *et al.*, 2014), kidneys (Adedara *et al.*, 2012), neuro-endocrine system and the gonads (Sherman and Sherman, 1979; Ortiz-Zarragoitia and Cajaraville, 2006).

Crude oil intoxication is known to induce tissue damage through oxidative stress including the generation of reactive oxygen species (Siegel *et al.*, 2004) by a non-enzymatic process (Bai *et al.*, 2001) or by cytochrome P-450 catabolic

Received: 30 Jun 2017. Received in revised form: 11 Jul 2017. Accepted: 19 Aug 2017. Published online: 30 Sep 2017.

enzymatic reactions (Ma *et al*., 2002). Earlier researchers demonstrated that exposure of male rats to crude oil adversely affected the testicular morphology, with the destruction of the connective tissues and Seminiferous tubules, leading to disruption of spermatogenesis and consequent oligospermia in the cauda epididymis (Akumka *et al*., 1999; Orisakwe *et al*., 2003; Orisakwe *et al*., 2004; Obidike, *et al*., 2007). Exposure of rats to crude oil consequently decreased serum levels of testosterone, progesterone, estradiol, Follicle stimulating hormone (FSH) and Luteinizing hormone (LH), while serum level of prolactin increased (Akaninwor and Okeke, 2006).

Vitamin E is a fat-soluble vitamin with high antioxidant potency. Vitamin E is a chiral compound with eight stereoisomers: α, β, γ, δ tocopherol and α, β, γ, δ tocotrienol. Only α-tocopherol is the most bioactive form in humans. Studies in both animals and humans indicate that natural dextrorotary d-α-tocopherol is nearly twice as effective as synthetic racemic dl-α-tocopherol (Nguyen *et al*., 2006). Vitamin E, also known as anti-sterility factor, possesses anti-oxidant activities (Uzunhisarcikli *et al*., 2007). This implies that vitamin E possesses the inherent potential to protect the reproductive tissues against many chemical as well as infectious pathologies (Pham-Huy *et al*., 2008). Vitamin E supplementations increase the resistance of chicks against *Escherichia coli* (Heinzerling *et al*., 1994) and of lambs against *Chlamydia* infection (Stephens and Nockles, 1979). Pigs supplemented with vitamin E and selenium had increased phagocytic activity of the reticuloendothelial system (Heinzerling *et al*., 1994).

The objective of the study therefore was to determine the effects of vitamin E administration on crude oil-induced reprotoxicity in male rats, with respect to testosterone level, epididymal sperm reserve, testicular weight, serum biochemistry and electrolyte levels.

Materials and Methods

Acquisition of crude oil

The crude oil used in this study was Nigerian Qua Iboe Brent crude oil obtained from Exxon Mobil Producing Limited, Eket, Akwa-Ibom State of Nigeria.

Acquisition of vitamin E

The vitamin E was mixed tocopherols commercially sourced from Puritan's Pride Premium.

Animals

Forty-four male Wistar rats with average weight of 250 grams were used for the study. They were obtained from the Animal House Unit of the Department of Veterinary Obstetrics and Reproductive Diseases, University of Nigeria, Nsukka. Prior to the commencement of the experiments, the animals were acclimatized for a period of three weeks. They were kept in metal cages under room temperature, with 12-hour light and 12-hour dark cycle. Clean water and feed (Vital Growers feed, GCOML, Jos, Nigeria) containing 14.5% crude protein were supplied *ad libitum*. Protocols for this experiment were in accordance with the guidelines on the care and wellbeing of research animals (NIH, 1985) and was approved by the Ethics Committee, Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

Experimental design

The rats were initially divided into three groups (A, B and C) comprising of 12, 16 and 16 rats respectively. Group A represented the unexposed control, whereas groups B and C were exposed orally to 0.15 and 0.3 ml of crude oil respectively every other day for 28 days.

At the end of the 28 days, 4 rats were randomly and humanely sacrificed from each group for testicular allometric weight, histomorphological and cauda epididymal sperm reserve studies. Thereafter, each group was further divided into subgroups of 4 rats each, thus, giving rise to A1 and A2; B1, B2 and B3; C1, C2 and C3 subgroups, respectively. Group A1 composed of the unexposed control rats, whereas 0.15 and 0.3 ml of crude oil was withdrawn from groups B1 and C1 respectively. Group A2 composed of the unexposed rats were supplemented with 0.1 ml of Vitamin E, whereas groups B2 and C2 were supplemented with 0.1 ml of Vitamin E following the withdrawal of 0.15 and 0.3 ml of crude oil respectively. There was continuation of 0.15 and 0.3 ml of crude oil in groups B3 and C3 respectively. All the treatments were given orally every other day for another 28 days.

Specimen collection and analysis

At the end of 28 and 56 days respectively, four rats from each group were randomly selected and weighed. Blood samples were collected from the medial canthus (retrobulbar plexus) of the eye using plain microhaematocrit tube into sterile sample bottles without anticoagulants. Then serum was harvested by centrifugation at 3,000 rpm for 10 min and stored at -20 °C until serum testosterone assay, serum biochemistry and electrolytes tests which were carried out within 24 hours of sample collection.

Serum testosterone levels were determined by ELISA technique with strict adherence to manufacturer's instructions for testosterone assay (Monobind inc. Lake forest, CA 92630, USA). Serum Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were determined by Reitman-Frankel colorimetric method for in vitro determination of ALT and AST in serum (Reitman and Frankel, 1957), using the QCA ALT test kit (Quimica Clinica Aplicada, Spain). Total protein was determined by direct Biuret method (Lubran, 1978) for *in vitro* determination of total protein in serum. Serum cholesterol was determined by enzymatic colorimetric method (Allain *et al*., 1974) for *in vitro* determination of cholesterol in serum using a QCA enzymatic cholesterol test kit (Quimica Clinica Aplicada, Spain). Serum concentrations of K^+, Na^+ and Cl^- were determined on days 28 and 56 post-crude oil treatment using ion selective method (Fry and Taves, 1970).

The rats were humanely sacrificed by stunning. The cauda epididymides were carefully dissected out and extraneous tissues trimmed off for the determination of the epididymal sperm reserve using the standard haemocytometric method of Amann and Almquist (1961). The two testes were also collected from each rat and weighed immediately for the determination of the testicular allometric weight and histomorphology.

Statistical analysis

The computer software, statistical package for social sciences (SPSS) version 9.0 was used for the statistical analyses. The data generated were subjected to one-way analysis of variance (ANOVA) and the means separated using least significant difference (LSD). Differences in the means were considered significant at the probability values less than 5 % ($p \leq 0.05$). The results were presented as the mean plus or minus the standard error of the mean (SEM)..

Results

Effects of Vitamin E supplementation on serum testosterone level (STL), cauda epididymal sperm reserve (CESR) and testicular allometric weight (TAW) in male rats with crude oil-induced reprotoxicity

At the end of 28 days (Table 1), both the low dose and high dose oral administration of Nigerian Qua Iboe Brent crude oil caused a significant reduction ($p \leq 0.05$) in the serum testosterone levels (STL) and cauda epididymal sperm reserve (CESR) of the exposed rats when compared to the control. However, there was no significant difference ($p \geq 0.05$) in the testicular allometric weights (TAW) across the groups.

On the 56th day post-exposure, it was observed that crude oil withdrawal and vitamin E supplementation did not significantly improve ($p \geq 0.05$) the STL of the exposed rats. Similarly, there were no significant variations ($p \geq 0.05$) in the TAW. Vitamin E supplementation, but not crude oil withdrawal, significantly improved ($p \leq 0.05$) the CESR in the low dose and high dose subgroups (Table 1).

Effects of Vitamin E supplementation on serum biochemistry in male rats with crude oil-induced reprotoxicity

The results of the effects of vitamin E supplementation on the serum biochemistry were presented in Table 2. The results showed that at the end of 28 days post-exposure, the serum Alanine Aminotransferase (ALT) and Serum Aspartate Aminotransferase (AST) of the rats exposed to high dose crude oil were significantly higher ($p \leq 0.05$) than those of the control and low dose groups. The mean serum total protein (STP) of the low dose-exposed rats was found to be significantly higher ($p \leq 0.05$) than that of the high dose-exposed rats, but did not differ ($p \geq 0.05$) from that of the control group. The mean serum total cholesterol (STC) value of the low dose-exposed rats was significantly higher than those of the control and high dose groups.

However, by the 56th day of the study, it was observed that vitamin E supplementation significantly reduced ($p \leq 0.05$) the mean serum ALT value in the unexposed subgroups from 47.13 (2.83) to 36.80 (1.51) UI/L. But vitamin E supplementation, and crude oil withdrawal and continuation, did not cause significant differences ($p \geq 0.05$) in the ALT activities in the exposed subgroups. There were no significant variations ($p \geq 0.05$) in the AST and STC of all the subgroups. Crude oil withdrawal, but not vitamin E supplementation, significantly reduced ($p \leq 0.05$) the mean STP values in the high dose subgroups only.

Table 1. Effects of Vitamin E supplementation on serum testosterone level (STL), cauda epididymal sperm reserve (CESR) and testicular allometric weight (TAW) in male rats with crude oil-induced reprotoxicity

Day 28 Post-Exposure			
Groups	STL (ng/ml)	CESR ($x10^6$)	TAW (g)
A (Control)	20.53 ± 5.24^{a}	110.00 ± 15.00^{a}	0.0117 ± 0.001
B (Low dose)	3.69 ± 2.77^{b}	48.00 ± 14.00^{b}	0.0111 ± 14.00
C (High dose)	4.92 ± 2.76^{b}	43.00 ± 11.00^{b}	0.0109 ± 0.001
Day 56 Post-Exposure/Supplementation			
Groups	STL (ng/ml)	CESR ($x10^6$)	TAW (g)
A1	10.20 ± 0.88^{a}	250.00 ± 19.00^{ab}	0.0103 ± 0.001^{ab}
A2	8.59 ± 1.34^{ab}	280.00 ± 21.00^{a}	0.0110 ± 0.000^{a}
B1	5.83 ± 1.73^{bc}	140.00 ± 31.00^{cd}	0.0093 ± 0.000^{abc}
B2	3.73 ± 0.68^{c}	200.00 ± 8.82^{bc}	0.0086 ± 0.000^{bc}
B3	2.65 ± 0.69^{c}	120.00 ± 27.00^{d}	0.0106 ± 0.008^{ab}
C1	4.25 ± 1.55^{c}	85.00 ± 33.00^{de}	0.0074 ± 0.001^{c}
C2	2.95 ± 0.66^{c}	130.00 ± 13.00^{d}	0.0074 ± 0.001^{c}
C3	2.89 ± 0.54^{c}	37.00 ± 5.06^{e}	0.0088 ± 0.000^{abc}

Superscripts in a column represent significant differences between groups' means ($p \leq 0.05$)

Table 2. Effects of Vitamin E supplementation on serum biochemistry in male rats with crude oil-induced reprotoxicity

Day 28 Post-Exposure				
Groups	ALT (IU/l)	AST (IU/l)	STP (mg/dl)	STC (mg/dl)
A (Control)	32.23 ± 2.06^{b}	74.43 ± 4.89^{b}	6.96 ± 0.22^{ab}	76.82 ± 0.47^{b}
B (Low dose)	32.09 ± 2.87^{b}	75.79 ± 1.99^{b}	7.27 ± 0.36^{a}	98.79 ± 9.26^{a}
C (High dose)	41.47 ± 7.78^{a}	82.08 ± 6.28^{a}	6.50 ± 0.25^{b}	83.02 ± 9.04^{b}
Day 56 Post-Exposure/Supplementation				
Groups	ALT (IU/l)	AST (IU/l)	STP (mg/dl)	STC (mg/dl)
A1	47.13 ± 2.83^{a}	97.69 ± 3.11	7.77 ± 0.27^{a}	73.20 ± 3.22
A2	36.80 ± 1.51^{b}	92.60 ± 1.97	7.45 ± 0.31^{ab}	72.34 ± 2.01
B1	38.02 ± 2.80^{b}	89.84 ± 4.14	7.22 ± 0.32^{ab}	78.62 ± 0.63
B2	41.26 ± 6.46^{ab}	86.79 ± 1.81	6.77 ± 0.11^{bc}	73.90 ± 2.87
B3	37.88 ± 2.96^{b}	97.70 ± 7.43	7.14 ± 0.03^{abc}	70.38 ± 6.79
C1	35.70 ± 1.36^{b}	95.59 ± 2.00	6.48 ± 0.37^{c}	80.35 ± 2.05
C2	40.15 ± 1.05^{ab}	90.11 ± 2.63	7.37 ± 0.18^{ab}	70.96 ± 10.76
C3	42.99 ± 1.87^{ab}	94.78 ± 3.50	7.58 ± 0.13^{a}	72.53 ± 4.26

Superscripts in a column represent significant differences between groups' means ($p \leq 0.05$)

Effects of Vitamin E supplementation on serum electrolytes in male rats with crude oil-induced reprotoxicity

Prior to vitamin E supplementation, the result in Table 3 showed that the mean serum K^+ and Cl^- concentrations did not vary ($p \geq 0.05$) between the unexposed and exposed groups, at the end of the 28 days. The mean serum Na^+ concentration of the low dose group however was found to be significantly higher ($p \leq 0.05$) than the mean serum Na^+ concentration of the high dose group, but not different ($p \geq 0.05$) from the mean serum Na^+ concentration of the control rats.

On the 56th day post-exposure, it was observed that continuation of high dose exposure of crude oil significantly increased ($p \leq 0.05$) the mean serum K^+ concentration when compared to the rest of the subgroups. Also high dose continuation as well as low dose crude oil withdrawal and vitamin E supplementation in the low dose subgroups significantly increased ($p \leq 0.05$) the mean serum Na^+ concentration. Vitamin E supplementation following low dose crude oil withdrawal significantly increased ($p \leq 0.05$) the mean serum Cl^- concentration.

Discussion

The current study revealed that exposure of male rats to both low dose and high dose Nigerian Qua Iboe Brent crude oil precipitated a reduction in the serum testosterone level (STL) which withdrawal and vitamin E supplementation could not reverse. This could be attributed to chronic or completely irreversible toxic changes induced by the crude oil in the male reproductive organs, especially the testicular tissues. This usually occurs due to endocrine disruption, thereby making the testicular tissues irresponsive to the gonadotrophic hormones. Against the general opinion that endocrine disruptors do not permanently alter the responsiveness of tissues to hormones, earlier experimental studies have shown permanent changes in the brain (Patisaul and Adewale, 2009), vaginal epithelium (Alder and Nelson, 1988; Ma, 2009) and prostate gland (Deklerk *et al*., 1979; Prins, 2008) following administration of oestrogenic chemicals to adult animals. This decline in the STL could also be as a result of massive catabolism of testosterone in the liver evoked by the crude oil exposure (Sayed *et al*., 2003).

There was also reduction in the cauda epididymal sperm reserve (CESR) of the rats exposed to low dose and high dose crude oil. The combined effects of the decrease in the serum testosterone level coupled with the degenerative changes in the epithelium of the seminiferous tubules associated with crude oil intoxication must have drastically reduced the population of the spermatozoa in the sperm depot of the tail of the epididymis (Obidike *et al*., 2007). Pflreger-Bruss *et al.* (2004) ealier reported disruption in testosterone output with the administration of crude oil to rats. Jewell *et al.* (1998) and Obidike *et al.* (2007) have also reported degeneration and necrosis of the spermatogenic cells of the germinal epithelium of the seminiferous tubules with attendant decline in the CESR, when rats were experimentally treated with crude oil and they attributed it to oxidative stress. However, crude oil withdrawal initiated while vitamin E administration potentiated the improvement in the CESR of the exposed rats. Vitamin E is an excellent primary component of the antioxidant system of spermatozoa (Wang *et al*., 2007), hence its administration significantly potentiated the improvement of CESR. Even though there was no change in the testicular allometric weight of the rats, it is noteworthy that spermatogenesis does not solely depend on the testicular size (Dalsenter *et al*., 1996).

The liver marker enzymes, ALT and AST are cytoplasmic in origin and are released into the circulation following cellular damage (Lin *et al*., 2000). The serum activities of ALT and AST have been reported to be increased as a result of liver injury in patients developing severe hepatotoxicity (Beckett, 1989; Tian *et al*., 2012). Chemical and organic toxicants like crude oil are known to cause liver damage and precipitate elaboration of ALT and AST above the basal level in the serum (Chang *et al*., 2013). The present study showed that a high dose oral administration of Nigerian Qua Iboe Brent crude oil is required to induce hepatotoxicity, leading to high activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) when compared to the low dose exposure and the control. After the induction of toxicity, vitamin E supplementation within 28 days was able to reduce the serum ALT activities in the unexposed, but not in the exposed subgroups. This could be traced to possible chronic damage to the hepatic tissues or delay in the degradation of the already produced enzymes (Mahmoud *et al*., 2011).

Leakage of proteins and even cholesterol into the extracellular compartment is an indication that the architecture of the cells is in disarray (Trimble and Grinstein, 2015). Exposure of rats to low dose of the crude oil increased the mean serum total protein (STP) and serum total cholesterol (STC) values as compared to the control and high dose of crude oil. This suggests that while low dose distorted the cellular architecture with consequent increase in the serum total protein (STP) and serum total cholesterol (STC), high dose of the crude oil probably incited a protective mechanism that maintained the serum total protein (STP) and serum total cholesterol (STC) within normal limits. Again, failure of vitamin E administration to reverse this aberration could be as a result of chronic or permanent changes in the cellular components (Trimble and Grinstein, 2015).

Finally, it was observed that oral administration of high dose of the crude oil lowered only the concentration of sodium ions, but vitamin E supplementation increased the concentrations of these electrolytes. This suggests that vitamin E was able to stabilize the membrane permeability for these electrolytes. Changes in ion transport and permeability are usually associated with inflammation due to auto-immune disease, infection and allergy, including chemical allergy (Eisenhut, 2006). Generally hydrocarbons, of which crude oil is one of them have been reported to be responsible for changes in membrane lipid composition, modification of outer membrane lipopolysaccharide, altered cell wall constituents, and active excretion of lipophilic ions thereby reducing their membrane concentrations (Sikkema *et al*., 1995).

Table 3. Effects of Vitamin E supplementation on serum electrolytes in male rats with crude oil-induced reprotoxicity

DAY 28 POST-EXPOSURE			
Groups	K^+ (mmol/l)	Na^+ (mmol/l)	Cl^- (mmol/l)
A (Control)	5.96 ± 0.04	142.52 ± 0.55^{ab}	103.69 ± 1.16
B (Low dose)	5.71 ± 0.22	142.89 ± 1.84^{a}	104.56 ± 1.87
C (High dose)	5.76 ± 0.16	140.53 ± 1.08^{b}	104.08 ± 1.29
DAY 56 POST-EXPOSURE/SUPPLEMENTATION			
Groups	K^+ (mmol/l)	Na^+ (mmol/l)	Cl^- (mmol/l)
A1	5.59 ± 0.27^{b}	134.29 ± 0.87^{ab}	104.91 ± 1.18^{b}
A2	5.86 ± 0.18^{ab}	132.65 ± 0.65^{b}	105.75 ± 0.20^{b}
B1	5.74 ± 0.18^{b}	136.78 ± 1.40^{a}	105.45 ± 0.70^{b}
B2	5.83 ± 0.09^{ab}	136.75 ± 0.91^{a}	108.90 ± 0.73^{a}
B3	5.51 ± 0.21^{b}	132.30 ± 1.28^{b}	107.17 ± 0.62^{ab}
C1	5.84 ± 0.05^{b}	134.85 ± 1.06^{ab}	104.53 ± 0.50^{b}
C2	5.63 ± 0.18^{b}	135.37 ± 0.85^{ab}	104.89 ± 1.21^{b}
C3	6.31 ± 0.12^{a}	135.82 ± 1.32^{a}	105.63 ± 1.76^{b}

Superscripts in a column represent significant differences between groups' means ($p \leq 0.05$)

Conclusions

The present findings showed that Nigerian Qua Iboe Brent crude oil induced serious reprotoxic effects in male rats, which vitamin E administration within 28 days did not completely reverse.

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