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Ameliorative effect of coconut oil (*Cocos nucifera*) on the testes of Norwegian rats intoxicated with untreated crude refinery effluents

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ABSTRACT

The study was aimed at determining the effects of untreated refinery effluent on the spermatogenic status of the testes in Norwegian rats and possible ameliorative effects of coconut oil. Thirty Norwegian rats were used for the study, divided into 3 groups of 10 animals each. Group 1 were given feed and drinking water, group 2 were given feed, drinking water and 2 mL of 100% of the untreated refinery effluent while group 3 were given feed, drinking water, 2 mL of 100% of refinery effluent and 2 mL of Coconut oil continuously for 9 weeks. At 3 weeks intervals, two rats were sacrificed from each group. Testicular tissues were harvested and analyzed. Treatment was discontinued after nine weeks. Groups 2 and 3 were re-designated groups 5 and 6, left for a period of 21 days without treatment. At the 21st day, testicular tissues were collected and analyzed. There was a significant increase ($P > 0.05$) in body and organ weight of treated rats compared to control. Organ morphology varied from control but was not statistically defined. Lead and chromium concentrations in testes were significantly different ($P > 0.001$) in effluent treated rats. Histopathology of the gonads showed evidence of pathologies in rats treated with effluent only compared to control and ameliorated groups. Testes of rats treated with coconut oil revealed normal spermatogenic architecture during and after exposure to refinery effluent. Coconut oil was effective in ameliorating the deleterious effects of untreated refinery effluent on the testicles of intoxicated Norwegian rats.

Keywords: Refinery effluents, toxicity, testis, coconut oil, amelioration.

Introduction

Humans are routinely exposed to many environmental pollutants such as pesticides, fertilizers, crude oil or any of its fractions on a daily basis. These pollutants have been implicated in many biochemical and toxicological effects on aquatic and terrestrial animals.[1] These substances can bio-accumulate in food chains and disrupt biochemical or physiological activities of many organisms, thus causing carcinogenesis of some organs, mutagenesis in the genetic material and impairment in reproductive capacity in exposed population.[2] Many chemical substances have been demonstrated to interfere with normal reproductive processes in several animal species.[2] One of such substances is Bonny Light Crude Oil (BLCO) which is a complex mixture of many different components. Crude oil, refined petroleum products, as well as polycyclic aromatic hydrocarbons are ubiquitous in various environmental compartments. Its exploration and transportation have generated a lot of environmental problems, especially in developing nations.[3] It is noteworthy, that the devastating consequences of crude oil spill in the Niger Delta of Nigeria pose great hazards on both aerial and terrestrial environments. Although, limited exposure to crude oil may also occur during drilling, transporting and refining, accidental spillage accounts for a more serious exposure to crude oil by wildlife and humans.[3] Furthermore, many of the people who live in the oil-rich areas are exposed to water from streams and ponds that have been polluted by oil spillage and dumping of untreated refinery effluents. This water is used for domestic activities such as drinking, cooking and washing by rural dwellers in the various oil-rich areas of the Niger Delta in South-South Nigeria. It is important to note that, majority of the people in the communities also ingest crude oil either directly as curative agents for anti-poisoning (snake venom antidotes), anti-convulsion, treatment of skin infection or indirectly by eating marine animals found in surrounding coastal waters as a source of protein.[4] BLCO is used in combination with olive oil in folklore medicine in some parts of the Niger Delta region of Nigeria to treat burns, gastrointestinal disorders, ulcers, witchcraft attacks and poisoning.[4, 5]

Although, various studies have been carried out on crude oil, very few have been directed at its impact on reproductive system. Increasing concern about the possible declining trend in fertility of man and wildlife animals over the past decades as a result of exposure to various environmental pollutants such as estrogenic agents and aromatic hydrocarbons have been widely reported.[3, 6] Previous studies showed that BLCO significantly reduced sperm count, sperm motility and normal morphology within seven days of administration [3, 5, 7]. There is paucity of information on whether or not this effect is capable of affecting fertility indices of these rats. Heavy metals found in petroleum refinery effluents such as chromium, lead, mercury, copper and zinc have received special attention in ecotoxicology in recent years.[8] Although, biological functions of organisms require some of these metals in trace amount but exposure to high concentration might be lethal or cause damages to cells and tissues in the body.[9] The therapeutic role of medicinal plants in ameliorating the toxicity effect of effluents and other contaminants has been widely reported.[10-12] Essential oils have also been utilized as therapeutic supplements since they are rich in biologically

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active compounds. Besides, coconut oil has been reported to have anticancer, antimicrobial, and anti-inflammatory properties.[13, 14, 15] A recent study reported that virgin coconut oil lowered alcohol-induced oxidative stress by reducing testicular malondialdehyde level (tMDA) and ameliorated the deleterious effect of alcohol on serum testosterone levels in rats.[15]

Coconut oil, a potent non-drug or natural yeast-fighter, contains three medium chain fatty acids, i.e., lauric acid (50–53%), caprylic acid, and capric acid, all of which have antifungal effect against *Candida spp* and other fungi. Coconut oil has been confirmed to possess antimicrobial, antiviral and antiprotozoal activities.[16]

Materials and Methods

Experimental Animals

This study was conducted in compliance with the Schedule Y guideline, Good Laboratory Practice (GLP) Regulations for Non-Clinical Laboratory Studies (21 CFR Part 58), OECD Good Laboratory Practice Principles (GLP) and in accordance with regulation of the Committee for the Purpose of Control and Supervision on Experiments on Animals No. 1568/RO/C/11/CPCSEA. All animal experiments were also conducted in accordance with standard guidelines on the use of animals for experimental toxicology study. Experimental animals were obtained from the animal house Unit of the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Nigeria.

Experimental Design

A total of thirty (30) male Norwegian rats weighing 108 ± 12 g were used for the experiment. Rats were randomly assigned into 3 groups of 10 animals each and allowed a period of 2 weeks to acclimatize before the start of the experiment. They were kept in wooden cages of dimension (3 m × 2 m ×1 m) with wire mesh covers. All rats were given feed and water *ad libitum*. Rats in group 1 served as the control group. Group 2 received 2 mL of 100% of the untreated refinery effluent *per os* continuously for 9 weeks; these served as the treatment group. Group 3 rats were given 2 mL of 100% of refinery effluent and 2 mL of coconut oil *per os* continuously for 9 weeks. At 3 weeks intervals, two rats were euthanized with chloroform from each group (1-3), samples (blood and testes) were harvested and analyzed for each experimental group till the end of phase 1. Treatment was discontinued after nine weeks. The remaining rats in groups 2 and 3 were designated groups 4 and 5. They were left alone for a period of 21 days without oral administration of both the untreated refinery effluent and the coconut oil. They were on feed and drinking water *ad-libitum*. This was the post-exposure stage of the experiment. At the end of the 21 days, samples (blood and testes) from each group (4 and 5) were collected and analyzed as well.

Refinery Effluent Collection

Refinery Effluent (Untreated waste water or produce water) was collected from a crude oil refinery; including both the tank farm drainage water and the spent caustic MEA (Monoethanolamine) from the refinery. This was then transferred to the laboratory in pre-cleaned 1.5 L plastic containers and stored at room temperature until use. These were considered as the stock effluent (100%).

Physical and Chemical Analysis of Refinery Effluent

Physical and chemical components of the untreated refinery effluent were analyzed and parameters such as pH, Temperature, Free Oil, Sulphides, Phenols, Total Nitrogen, Total alkalinity, Chemical oxygen demand (COD), Total suspended solids (TSS), Turbidity, and Dissolved solids, were determined. The concentrations of heavy metals namely; dissolved iron (Fe), lead (Pb), copper (Cu), manganese (Mn), chromium (Cr), Mercury (Hg), Arsenic (As) and Total Hydrocarbon Content (THC) were also determined.

Coconut Oil Preparation

Fresh coconut (*Cocos nucifera*) was obtained from the New Benin Market, Benin City, Edo State, Nigeria. The fresh coconut meat was grated and pressed using a sterilized sieve to produce coconut milk, which was allowed to ferment for 48 h, after which the solids and water content were separated from the oil. The oil was then heated in a water bath slightly to remove retained moisture. The oil was filtered by passage through a 25 m pore size filter (Millipore, St. Quentin, France) to give an aqueous extract of coconut oil. This was collected in a sterile vial and stored at 4°C until use.[17]

Phytochemical Analysis of Coconut Oil

Phytochemical analysis was carried out and tests were conducted to determine the presence of flavonoids, tannins, cardiac glycosides, saponin, steroids, terpenoids**,** alkaloids**,** and reducing sugar according to standard methods.[18]

Anti-microbial Analysis of the Effluent using Coconut Oil

Preparation of Culture media

All media were prepared according to manufacturer instruction. The media used in this study include nutrient agar and McConkey agar.

Isolation of Bacteria

Total viable heterotrophic bacterial counts were determined using pour plate technique. Then the molten nutrient agar at 45ºC was poured into the Petri dishes containing 1 mL of the appropriate dilution for the isolation of the total heterotrophic bacteria. They were swirled to mix and allowed to solidify. The nutrient agar plates were incubated at 37ºC for 24 h. Colony counts were taken after incubation, then recorded in colony forming unit per milliliter and preserved by sub culturing the bacterial isolates into nutrient agar slants which were used for biochemical tests.

Enumeration of microorganisms

The standard method for estimating bacterial and fungal counts was used to enumerate the total viable counts of the isolates.[19]

Sub-culturing of bacterial isolates

A single isolated colony of the bacteria was picked up with the help of sterilized wire loop and was streaked on fresh nutrient agar medium. The nutrient agar plates were incubated at 37ºC for 24 h. The isolated bacterial strains were refrigerated after preparing slants.

Characterization and Identification of Bacterial Isolates

The bacterial isolates from the effluents were identified based on standard microbiological methods.[20] Isolates were identified based on their macroscopic and microscopic characteristics with reference to standard identification keys and atlas.[21]

Physical Observations, Body and Organ Weight Measurement

Each rat in each of the treatment groups was observed twice daily (before and after exposure) for signs of clinical toxicity in the appearances of the skin and fur, eyes and mucous membrane, behavioral pattern, respiratory system, morbidity and mortality. The body weight of each animal in the control and treatment groups was measured at the beginning of the experiment and at the end of exposure period using OHAUS® Scout ™ Pro, Model: SPU202 digital weighing balance. The testis of the animals were surgically removed and weighed, then bottled with formal saline and Nitric acid depending on the analysis. The Absolute organ weight was determined.

Heavy Metal Concentration Analysis

Metal concentrations in the organs were determined by atomic absorption spectrophotometry as previously described by Brzoska.[22] The model used was the Buck Scientific 210 VGP.

Histopathology

The fixed testes were dehydrated in ascending concentrations of alcohol, cleared in xylene for 90 min, and embedded in paraffin wax. Sections 5 microns thick were made and mounted on slides. The slides were stained with hematoxylin, counter stained with eosin (H & E stains) and viewed under an Olympus Microscope (light microscope) (Nikon Eclipse E400). All alterations from the normal structure were registered. Photomicrographs were obtained at different magnification to show the differences in tissues of the testes of the rats for each experimental group at varying time phase.

Statistical Analysis

All data were analyzed using the Statistical software, SigmaPlot for advance statistics (Systat Inc. USA, 2010), Version 12.0. Significant difference between treatment(s) and control was analyzed with Two-Way and One-way ANOVA (Analysis of Variance) under DMR test (Duncan Multiple Ratio) for pair-wise comparison to detect significant differences at $P \leq 0.05$.

Results and Discussion

Coconut oil which exhibited ameliorative effect on antispermatogenic effect induced by refinery effluent was found to be rich in essential constituent that could serve as anti-oxidants and also useful in nutrient enrichment. Flavonoids, terpenoids, steroids, anthraquinones, saponins, reducing sugars, alkaloids, tannins and cardiac glycosides were detected in both substances.

During the duration of exposure of rats to the effluents, increased water intake was observed in rats across the treatment groups compared to control. After 2 weeks of exposure, there was a continued increase in body weight across all groups (Table 1). At the end of the sixth week, rats in the control group and those given coconut oil were more active. Other observations such as diarrhea, loss of fur, swelling of the body and loss of appetite were also observed in refinery effluent treated rats.

Body weight of rats estimated at 3, 6 and 9 weeks of treatment increases across all treatment groups (Table 2). There was also an increase in body weight of rats treated for 9 weeks before the 21-day post-treatment. From week 10 to 13 post-treatment, there was a decrease in body weight of rats treated with effluent only (Figure 1). Treated rats given coconut oil showed increase in weight gain after week 10-13 (Figure 2). Rats treated with effluent and coconut oil as abatement increases in body weight from week 10 to 11 but decrease from week 11 to 12 with a slight increase in body weight observed from week 12-13 (Figure 2). The urinalysis of some selected rats at the end of 6 weeks of exposure and after the 21-day recovery period showed that the pH reduced in all the groups except in the group given coconut oil. After 21 days, mean pH was 8.0 in all groups.

Testes were found to contain certain detectable concentrations of chromium and lead, the Chromium (Cr) and lead (Pb) concentration were found to be significantly different ($P < 0.001$) in rats treated with effluent as compared to those that also received coconut oil (Table 3). The maximum chromium and lead concentrations found in the gonads were in the effluent treated rats. In the testes, the highest concentration of chromium in effluent treated rats (slightly > 0.018 mg/kg) was observed at 3 weeks post-treatment while for lead, the highest concentration (0.9 mg/kg) was observed at the 21 days post-treatment period (Figure 3).

At 3 weeks of exposure; the testes of rats in group 1 appeared normal in shape and morphology with no visible sign of degeneration composing of seminiferous tubules and interstitial space (Figure 4). The testes of rats treated with effluent only showed mild interstitial congestion and testicular edema (Figure 5). At 6 weeks of exposure; the testes of rats in the control group appeared normal in shape and morphology with no visible signs of degeneration, visible from the histology of the seminiferous tubules and interstitial space (Figure 7). The testis of the male rat given effluent only showed patchy spermatogenic arrest (Figure 8). The testes of rats treated with effluent and coconut oil showed moderate interstitial edema (Figure 9). At 9 weeks of exposure; the testis of the rats given effluent only showed vascular degeneration and spermatogenic arrest (Figure 10). The testis of the rats given effluent and coconut oil were normal (Figure 11). After 21 days recovery period; the testis of rats in which effluent only was discontinued showing mild spermatogenic arrest (Figure 12). While the testis of the male rats in which effluent and coconut oil were discontinued showing normal maturation and complete return of spermatogenesis stages moderate lymphoid activation (Figure 13).

The development of the oil industry has generally made the environment particularly the wetland ecosystem vulnerable to damaging effects of oil pollution. Contamination of aquatic environment by effluents from petroleum industries constitutes a source of stress to aquatic organisms. Studies have shown that exploratory activities and downstream conversion in refinery generate several pollutants and chemicals to surrounding water bodies[23] and these activities cause toxicological effects on aquatic lives [23, 24] and also mammals that are directly or indirectly exposed to the contaminants.

In this study, significant increase in body weight throughout the duration of exposure was observed in rats treated with refinery effluent and also in those that received coconut oil. In the period when rats were withdrawn from treatment i.e. post-treatment period, significance reduction in body weight across the treated groups was equally observed (Table 4). Though, it has been reported that rats exposed to industrial effluents show a significant reduction in their body weight on exposure; this study found an increase in body weight of rats upon exposure and rats during the exposure period showed no obvious sign of loss of appetite. The increased body weight might be an indication of toxicity via accumulation of fluids in organs and body tissues and deposition of heavy metals in tissues. Studies have reported weight gain in some organs of rats on exposure to contaminants such as leachates. The authors attributed it to heavy metals in the contaminant since these organs are involved with sequestration of metals. [5, 25] However, other studies had shown reduction in body and organ weight of rats exposed to textile effluent at concentrations that was reported toxic [26] This study also reported an increase in organ weight of treated rats with reduction in morphology such as length. Increase in organ weight e.g. testes weight is often an indication of gonadal pathology. The testes of the rats in the treatment and ameliorated group showed distinct signs of inflammation (congestion and edema). This could have been due to the deleterious effects of acute lead intoxication. The amelioration seems not to have been effective at 3 weeks. There was some degree of spermatogenic arrest after 6 weeks which was indicated by alterations of the normal testis architecture. In agreement with this, a study reported that the testes of rabbits given a dose of 200 mg of lead showed deformities in the architecture with serious damage within the seminiferous tubules.[27] These damages were however abated from the 6th week of amelioration with coconut oil. Some degree of spermatogenic arrest however continued at the 9th week in the treated group which was absent in the ameliorated group. At the end of the 21 days post exposure period, the spermatogenic arrest seems to have been reduced with visible signs of return to normal testicular architecture. Moreover, more works should be done in order to ascertain which of the heavy metals in the effluents specifically had anti-spermatogenic effects on the testes.

Coconut oil has been reported to contain polyphenols [28, 29] and polyphenols have been reported to possess various biological actions, including anti-nociceptive and anti-inflammatory activities,[30, 31] effect on signal transduction, activation of pro-inflammatory transcription factors and gene expression [32, 33] this may account for the protective effect on the testes of the rats in this study and the non-detectable levels of heavy metal accumulation in the testes.

Coconut oil had inhibitory action against the six bacteria isolates (*Staphylococcus aureus*, *Micococcus letus*, *Bacillus subtilis*, *Klebsiella, pneumonia Escherichia coli* and *Pseudomonas aeruginosa*) from the refinery effluent. (Figure 14). Similar results of anti-microbial activity of essential oil on *E. coli*, *S aureus* has also been reported; extract of coconut (*Cocus nucifera*) has a high inhibitory activity against *S. aureus*, *P. aeruginosa*, *Klebsiella pneumonia*, *E. coli*, *Micococcus letus* [34]

This study has provided adequate information on the toxic effects of untreated refinery effluent on the testicles of Norwegian rats. It has also highlighted the benefits of coconut oil as a possible agent in ameliorating the deleterious effects of refinery effluent toxicity. Histological examinations revealed varying pathological changes, tubular necrosis, spermatogenic arrest and interstitial edema. However, the use of coconut oil ameliorated further damage to testicular tissues resulting from the exposure to untreated refinery effluent.

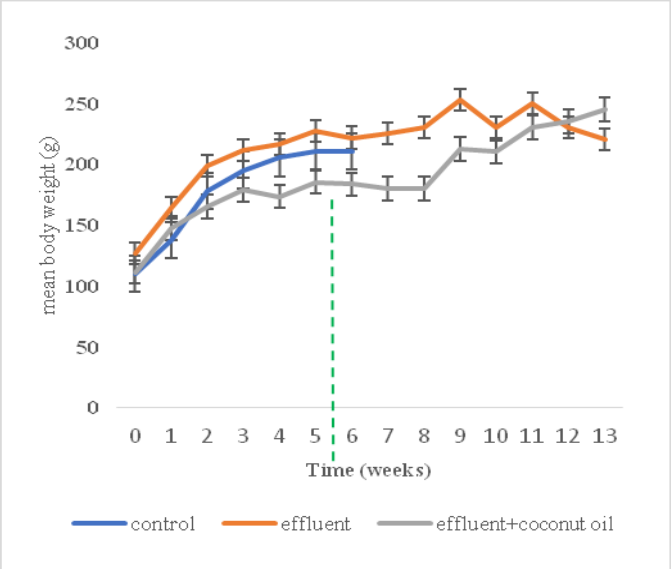

Figure 1: Changes in mean body weight of rats across the experimental groups at various time points.

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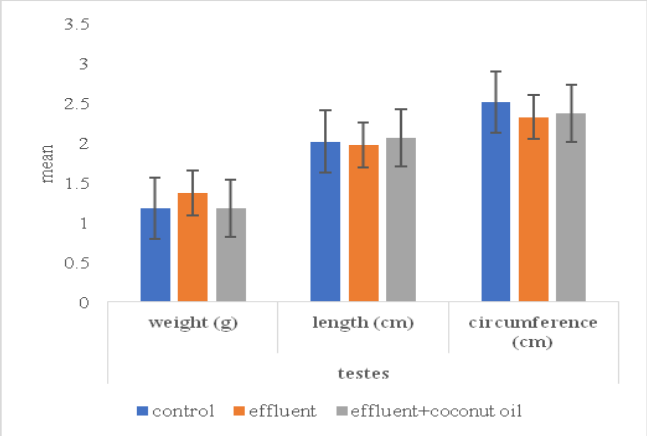

Figure 2: Mean weight, length and circumference of testes across experimental groups.

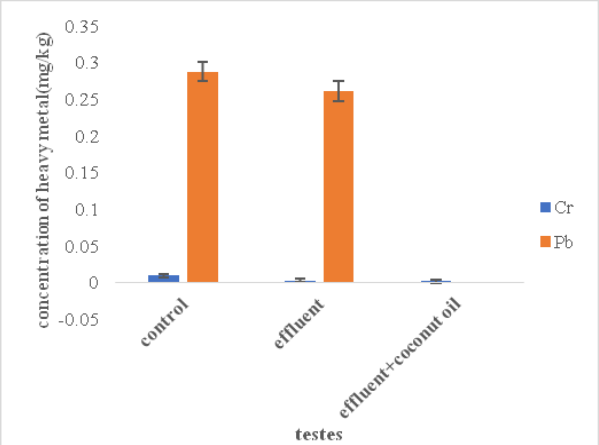

Figure 3: Concentration of heavy metals lead and chromium in the testes across experimental rats.

Table 1: Mean body weight in male subjects across the control, effluent and coconut oil abated groups and at various time points

Time period (week)	Group	Time point	Mean body Weight (g)	SE (Standard Error)
1-3	1	0	110	12.5
		1	137.5	12.553
		2	177.5	14.835
		3	195	17.191
	2	0	126.25	8.839
		1	163.75	8.877
		2	198.75	10.49
		3	211.25	12.156
	3	0	111.25	8.839
		1	147.5	8.877
		2	165	10.49
		3	178.75	12.156
4-6	1	0	115	16.833
		4	205	11.547
		5	210	10.206
		6	210	16.833
	2	0	118.333	9.718
		4	216.667	6.667
		5	226.667	5.893
		6	221.667	9.718
	3	0	106.667	9.718
		4	173.333	6.667
		5	185	5.893
		6	183.333	9.718
7-9	2	0	115.5	7.906
		7	225.0	5.303
		8	230.0	7.017
		9	253.0	8.839
	3	0	95.0	7.906
		7	180.0	5.303
		8	180.0	7.017
		9	212.5	8.839
10-13	5	10	230	
		11	250	
		12	230	
		13	220	
	6	10	210	
		11	230	
		12	235	
		13	245	

Table 2: Changes in mean organ weight in rats across control, effluent and coconut oil abated groups.

Organ	Measure	Group	Mean	SD	*P*
Testes	Weight (g)	1	1.1775	0.0789	0.025
		2	1.3663	0.19316	0.038
		3	1.1725	0.08242	
	Length (cm)	1	2.015	0.01915	0.814
		2	1.9712	0.40717	
		3	2.0588	0.11051	
	Circumference (cm)	1	2.51	0.5889	0.878
		2	2.3213	0.53485	
		3	2.3713	0.67558	

Table 3: Results of One-way Analysis of Variance for mean concentration of Chromium and Lead in the body organ across various experimental groups.

Organ	Metal	Group	Mean	SD	*P*
Testes	Chromium	1	0.009	0.012728	0.257
		2	0.0025	0.00238	
		3	0.00105	0.001418	
	Lead	1	0.2875	0.19445	0.491
		2	0.2608	0.42972	
		3	0.034	0.03879	

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Table 4: Results of repeated measures ANOVA and test of significance of mean body weights in male subjects between various time points across the control, effluent and coconut oil abated groups.

Weight	Time period (week)	Time point	Mean body weight (g)	SE	Compared time points	Mean difference	*P*
Body	1 - 3	0	115.833	5.893	0 - 1	-33.75	<0.0001
		1	149.583	5.918	0 - 2	-64.583	<0.0001
		2	180.417	6.993	0 - 3	-79.167	<0.0001
		3	195	8.104	1 - 2	-30.833	<0.0001
					1 - 3	-45.417	<0.0001
					2 - 3	-14.583	0.003
	4 - 6	0	113.333	7.244	0 - 4	-85.0	<0.0001
		4	198.333	4.969	0 - 5	-93.889	<0.0001
		5	207.222	4.392	0 - 6	-91.667	0.001
		6	205	7.244	4 - 5	-8.889	0.486
					4 – 6	-6.667	>0.05
					5 - 6	2.222	>0.05
	7 - 9	0	105	5.59	0 - 7	-96.25	0.041
		7	201.25	3.75	0 - 8	-100	0.048
		8	205	5.0	0 - 9	-118.75	0.056
		9	223.75	6.25	7 - 8	-3.75	0.573
					7 - 9	-22.5	0.177
					8 - 9	-18.75	0.226
	10 - 13	10	220.0				
		11	240.0				
		12	232.5				
		13	232.5				

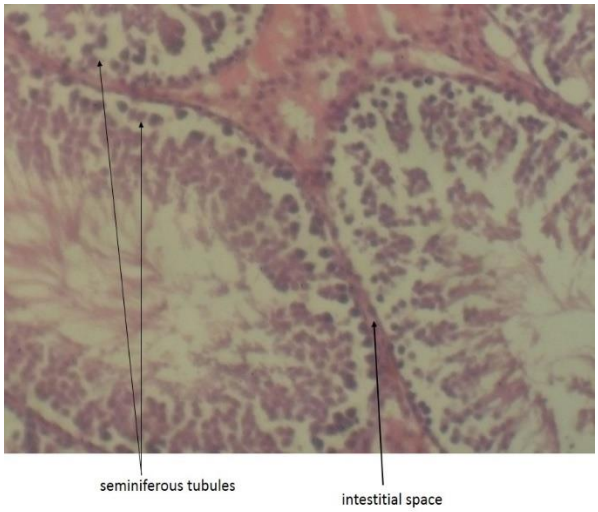

Figure 4: Testis of control rat at week 3 showing normal seminiferous tubules and interstitial space (H&E x 100).

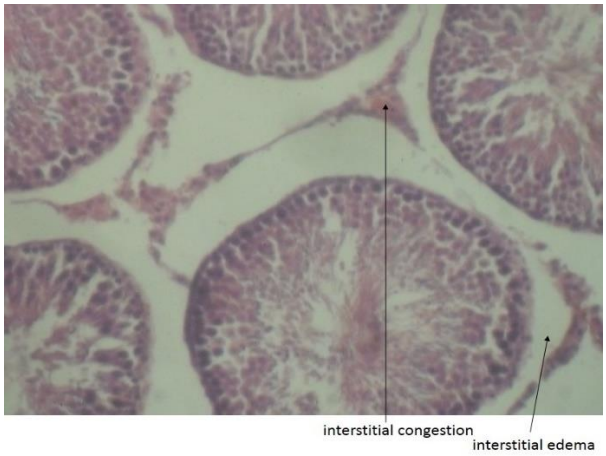

Figure 5: Testis of rat treated with refinery effluent only at week 3 showing interstitial congestion and edema (H&E x 100).

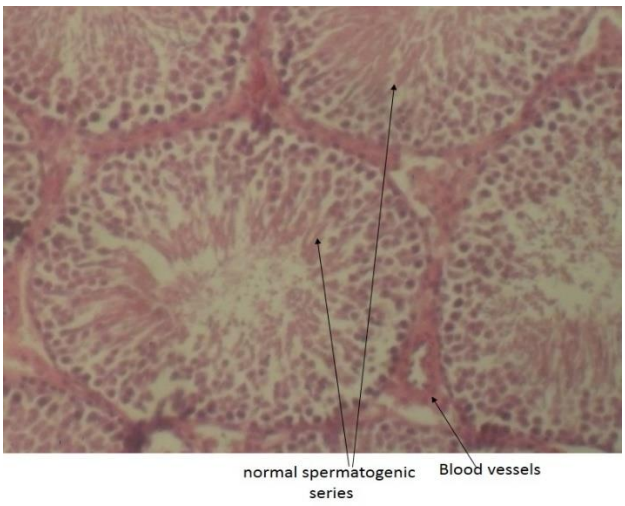

Figure 6: Testis of rat given effluent and coconut oil at week 3 showing normal spermatogenic series and blood vessels (H&E x 100).

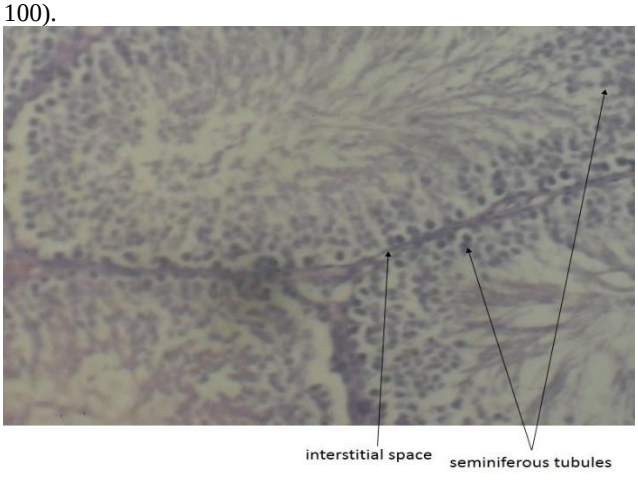

Figure 7: Testis of control rat at week 6 composed of normal seminiferous tubules and interstitial space (H&E x 100).

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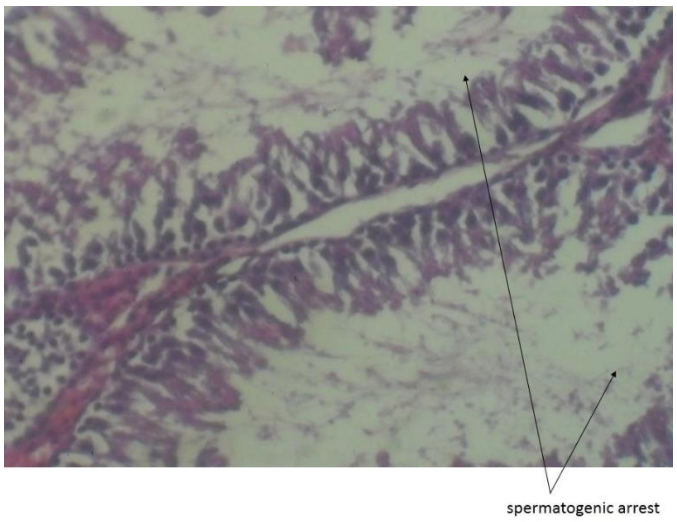

Figure 8: Testis of rat treated with refinery effluent only at week 6 showing spermatogenic arrest (H&E x 100).

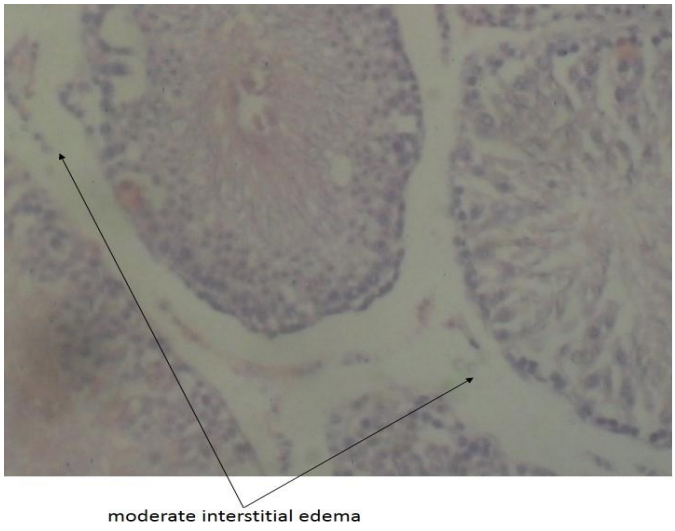

Figure 9: Testis of rat given effluent and coconut oil at week 6 showing moderate interstitial edema (H&E x 100).

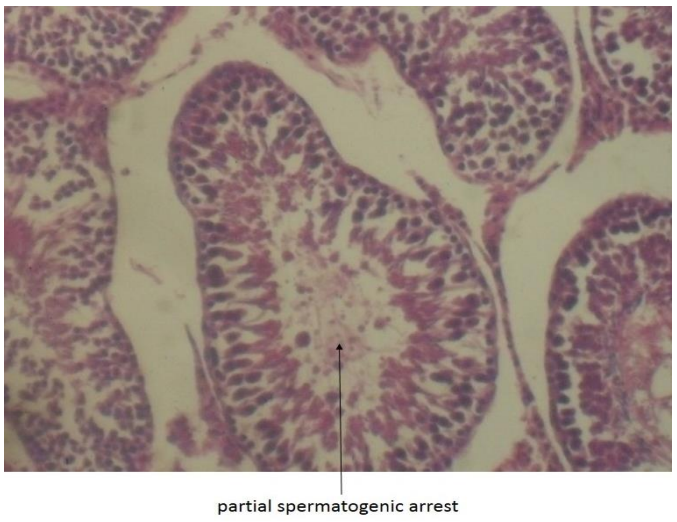

Figure 10: Testis of rat given refinery effluent at week 9 patchy spermatogenic arrest (H&E x 100).

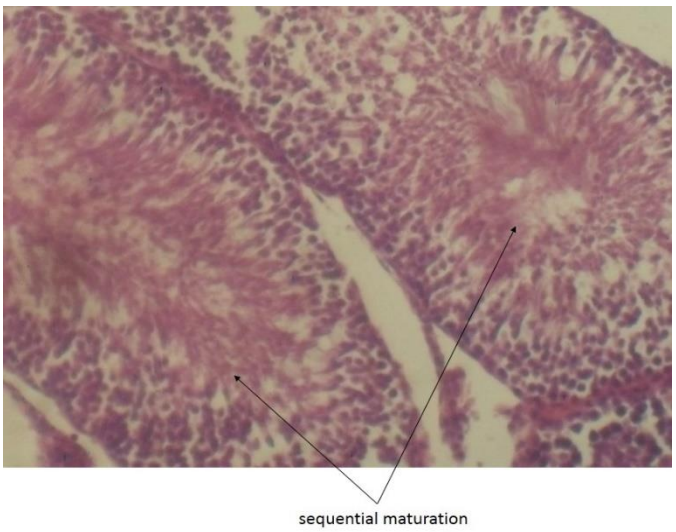

Figure 11: Testis of rat given effluent and coconut oil after 21 days resting period showing normal sequential spermatogenic maturation (H&E x 100).

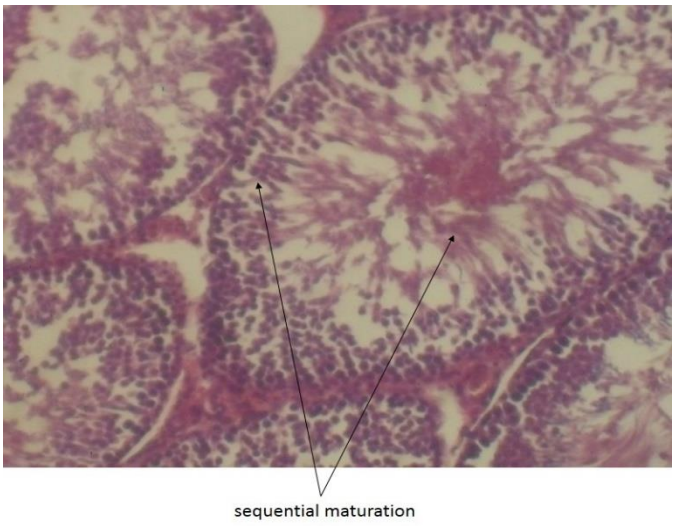

Figure 12: Testis of rat given effluent only after 21 days resting period showing normal sequential spermatogenic maturation (H&E x 100).

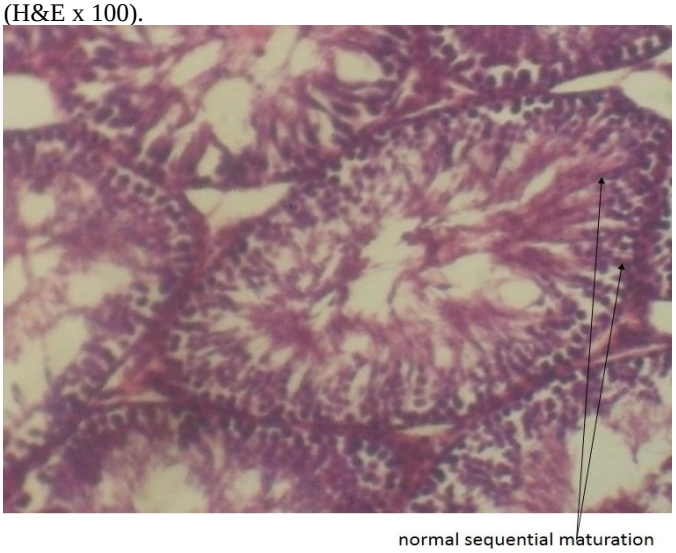

Figure 13: Testis of rat given effluent only after 21 days resting period showing normal sequential spermatogenic maturation (H&E x 100).

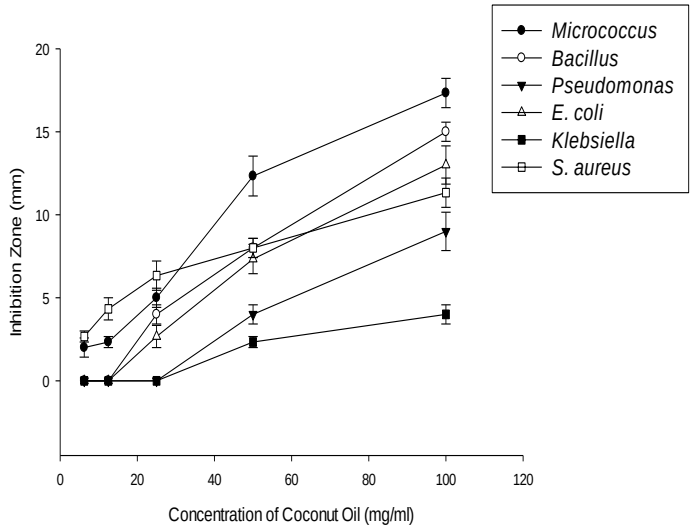

Figure 14: Microbial inhibition zone of coconut oil for positive culture organisms in refinery effluent.

Conclusion

From this study, exposure to refinery effluents resulted in spermatogenic arrest in Norwegian rats and coconut oil had ameliorative effects on the spermatogenic arrest resulting from refinery effluent toxicity. Coconut oil offered a measure of protection to the testes of Norwegian rats intoxicated with refinery effluents. Based on the findings from this study, it is recommended that industrial waste such as refinery effluent should be properly treated prior to release into the environment, there should be regular regulatory assessment of the treated effluent using appropriate risk assessment procedures, and there should be strict regulation on the release of industrial effluents into the environment.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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