PRELIMINARY STUDIES ON THE PROTECTIVE EFFECT OF SHEA OIL ON THE GONADS OF MALE NORWEGIAN RATS INTOXICATED WITH UNTREATED REFINERY EFFLUENTS

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ABSTRACT

This study reports the possible ameliorative effect of Shea oil on the testes of Norwegian rats intoxicated with untreated refinery effluent. 3 groups of 10 animals each were treated with Effluent only, Effluent with Shea oil and an untreated control daily for 9 weeks. Rats were euthanised at 3, 6 and 9 weeks interval. After 9 week, rats were left for 21 days post-exposure to recuperate. Body weight, testicular weight and morphology, heavy metal concentration in testes and histopathology of testes were estimated across the groups. There was a significant increase (p>0.05) in body and organ weights of treated rats compared to control. Gross organ morphology also varied widely between treated and control but this was not statistically significant. Lead and chromium concentrations in the testes were high in rats treated with effluent only. Histopathology of gonads also showed evidence of pathological effects in rats treated with effluent only as compared with control and ameliorated group. Rats treated and ameliorated with Shea oil showed normal histological architecture in their organs. The study concluded that Shea oil was effective in ameliorating the effects of untreated refinery effluent toxicity on the gonads of male rats.

Keywords: Shea oil, Refinery effluents, Testis, Amelioration

INTRODUCTION

A myriad of industrial activities cause environmental pollution, industries such as refinery and agrochemical industries contribute to the degradation of the environment as they release effluent that are toxic into the environment. According to the United State Environmental Protection Agency (USEPA), effluent (treated or untreated) generated from industrial plants are discharged to water bodies; with effluent from petroleum refinery been reported to be a major source of aquatic pollution (Wake, 2005). Refinery effluent majorly consist of several organic and inorganic compounds including phenolic groups, organochlorides, grease and oil and also heavy metals such as chromium, lead, mercury and cadmium (Diya'uddeen *et al.,* 2011). Thus, the effect of petroleum refinery effluent on the environment is severe as; they are widely distributed and pose environmental and occupational exposure risks, which may result in adverse health effects to man (Permenter *et al*., 2011). Exposure to effluent that contains heavy metals may occur through direct exposure to contaminated substrate such as soil, air, water and food or via consumption of aquatic lives through uptake and biomagnification (Marouani *et al*., 2011).

Heavy metals found in petroleum refinery effluent such as chromium, lead, mercury, copper and zinc have received special attention in ecotoxicology (Guertin, 2005). Although, the biological functions of organisms require some of these metals in trace amount, exposure to high concentrations might be lethal or cause damages to cells and tissues in the body (Cohen *et al*., 1993).

It has been reported that exposure to chromium (VI) causes reproductive toxicity in human and laboratory animals (Danadevi *et al.,* 2003; Subramanian *et al*., 2006). Furthermore, a decreased concentration of sperm cells and increase in abnormal spermatozoa were observed in Swiss mice after chromic acid exposure (Acharya *et al.,* 2006), in rats (Marouani *et al*., 2017), in rabbits exposed to chromium (Yousef *et al*., 2006) and bonnet monkeys that were equally exposed to chromium (Subramanian *et al*., 2006).

The therapeutic role of medicinal plants in ameliorating the toxicity effect of effluent and other contaminants has been widely reported (Chatterjee *et al*., 2012; Adikwu *et al*., 2013; Ugwu *et al*., 2013). Essential oils are used as therapeutic supplements because they are rich in biologically active compounds. Shea butter oil, extracted from Shea tree (*Vitellaria paradoxa)* reportedly possesses antimicrobial, antifungal, antiviral and insecticidal properties (Kordali *et al*., 2005). About 85 to 90 % of the fatty acid composition is stearic and oleic acids. A study had characterized and quantified the most important phenolic compounds in Shea oil (Maranz *et al*., 2004). Phenolic compounds that are a major constituent of Shea oil are known to have antioxidant properties against tissue damage. Assessment of the toxic effect of effluent have been carried out using animal models such as the Norwegian rat (*Rattus norvegicus)* in order to extrapolate the toxic effect of these harmful substances and compounds in humans (Isselhard and Kushe, 1998; Giridharan *et al*., 2000; Ihedioha *et al*., 2004).

For a clearer insight into the effects of exposure to untreated refinery effluent and its wider effect on the environment, an assessment on the toxicity response of *Rattus novergicus* exposed to untreated refinery effluent with possible abatement using Shea oil was carried out. The specific aims of this study therefore were to; determine the level of heavy metal concentration on the testicles of the rats and observe the histopathological alteration in the testes of the treated rats. The study also evaluated the possible ameliorative effect of Shea oil on intoxicated rats and determined if rats can recuperate over a short period of time post exposure to effluent.

MATERIALS AND METHODS

Experimental Design: Thirty adult male albino rats were used for the study. Rats were grouped randomly into three groups of 10 animals each. Rats in Group 1 were given feed and drinking water *ad-libitum* all through the experiment. Group 2 rats were given feed and drinking water *ad-libitum* and 2 ml of 100 % untreated refinery effluent daily for 9 weeks *per os*. Group 3 rats were given feed and drinking water *ad-libitum*, 2 ml of 100 % of refinery effluent and 2 ml of Shea oil 1 hour after, daily for 9 weeks. Every three weeks, two rats were euthanized from each group (1 – 3). Blood and testicular tissue samples were collected and analysed. Treatment was discontinued after nine weeks. The remaining rats in groups 2 and 3 were designated groups 4 and 5. They were untreated for additional three weeks (representing weeks 10 – 12 recovery period), but given food and drinking water *ad-libitum*. Blood and testicular tissue samples from groups 4 and 5 were collected and analysed after this post exposure phase as well.

Refinery Effluent Collection: Refinery effluent was collected from a crude oil refinery this included both the tank farm drainage water and the spent caustic and MEA (monoethanolamine) transferred to the laboratory in pre-cleaned 1.5 litre plastic containers and stored at room temperature until use. This was the stock effluent (100 %).

Physical and Chemical Analysis of Refinery Effluent: Physical and chemical components of the untreated refinery effluent were analysed

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 tetrahydrocannabinol THC were also determined using APHA (2005).

Phytochemical Analysis of Shea Oil: Phytochemical analysis was carried out to determine the presence of flavonoids, tannins, cardiac glycosides**,** saponin, steroids**,** terpenoids**,** alkaloids and reducing sugar in Shea oil according to the methods in Harborne (1989).

Anti-Microbial Analysis of the Effluent and Shea Oil

Preparation of culture media: All media were prepared according to manufacturer instruction. The media used in this study include nutrient agar and MacConkey agar.

Isolation of bacteria: Serial dilution of each sample was made to 10^{-1}, 10^{-2} and 10^{-3} dilutions. Total viable heterotrophic bacterial counts were determined using pour plate technique (Taylor *et al.,* 1983). Colony counts were taken and recorded in colony forming unit per millilitre.

Enumeration of microorganisms: Total viable counts of bacterial and fungal isolates were enumerated using the standard methods for estimating bacterial and fungal counts (Collins *et al*., 1989).

Characterization and identification of bacterial isolates: The bacterial isolates were identified based on microbiological methods and cultural characteristics and the fungal isolates were identified based on their macroscopic and microscopic characteristics with reference to standard identification keys and atlas (Cowan, 1985).

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 skin and fur, eyes and mucous membrane, behavioural pattern, respiratory rates, morbidity and mortality. The body weights of each animals in the control and treatment groups were measured at the beginning of the experiment and at the end of exposure period using a digital weighing balance (OHAUS® Scout™ Pro, Model: SPU202). The testes of the animals were surgically removed, weighed to measure the absolute organ weight, and fixed in Bouins fluid for histological analysis using the method of Waheed and Ansari (2012).

Heavy Metal Concentration Analysis: Metal concentrations in the testicles was determined using atomic absorption Spectrophotometer (Buck Scientific 210 VGP) as described by Brzoska *et al.* (2002).

Histopathology

Testicular histopathology: Testes of each rat fixed in Bouins fluid, were passed through ascending series of ethanol and then through xylene and embedded to paraffin wax. The tissues were sectioned at 15 µ, stained with Haematoxylin and Eosin, and mounted. All sections were examined under light microscope (Nikon Eclipse E400) in x100 and x400 magnifications. Photomicrographs of the lesions were taken for observation and documentation of histopathologic lesions. All alterations from the normal structure were registered.

Statistical Analysis: All data were analysed using the statistical software, Sigma Plot version 12.0. Significant difference between treatment(s) and control were analysed using analysis of variance (ANOVA). Variant means were separated using Duncan's New Multiple Range Test. Significant differences at $p \leq 0.05$ were accepted.

RESULTS

Phytochemical Analysis of Shea Oil: Shea oil was found to be rich in essential constituent that could serve as anti-oxidants and useful in nutrient enrichment. Flavonoids, terpenoids, steroids, anthraquinones, saponin, reducing sugar, alkaloids, tannin and cardiac glycosides.

Body Weight: There was a significant variation ($p<0.05$) in the body weight of rats treated for 9 weeks (Figures 1 – 4) across the three groups. From week 10 to 12 recovery period, there was a significant decrease ($p<0.05$) in body weight gain of rats treated with effluent only and in rats treated with effluent and Shea oil (Figure 1).

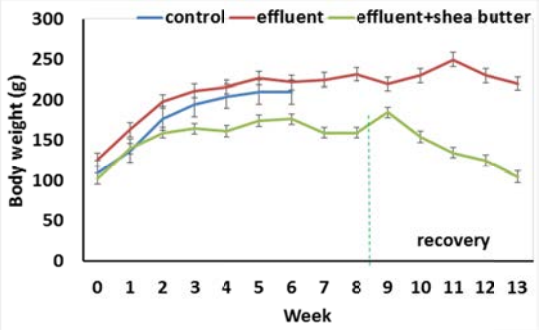

Figure 1: Changes in mean body weight of control, effluent treated and Shea oil ameliorated male rats from week 0 – 13 respectively

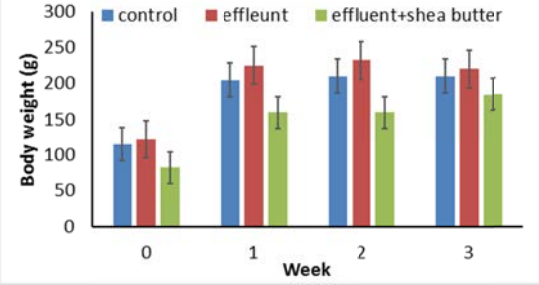

Figure 2: Changes in mean body weight of control, effluent treated and Shea oil ameliorated male rats at week 0 – 3 respectively

Testicular Weight and Morphology: There was no significant difference ($p>0.05$) in the weight of testes across all the treatment groups at the end of the 9th week of test duration (Figure 5).

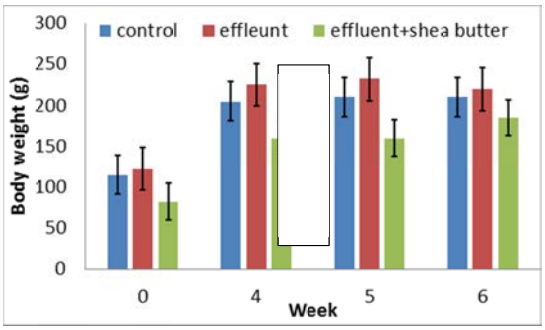

Figure 3: Changes in mean body weight of control, effluent treated and Shea oil ameliorated male rats at week 4 – 6 respectively

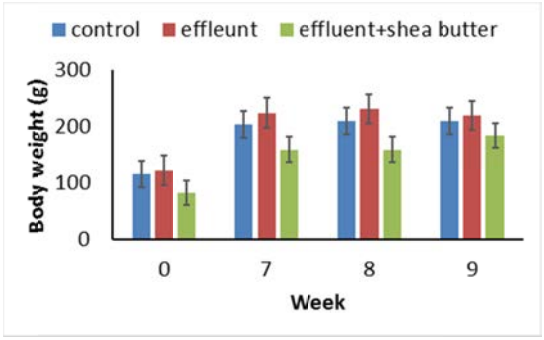

Figure 4: Changes in mean body weight of control, effluent treated and Shea oil ameliorated male rats at week 7 – 9 respectively

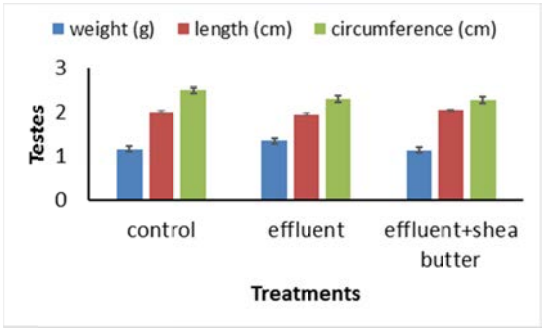

Figure 5: Changes in mean weight, length and circumference of testes of control, effluent treated and Shea oil ameliorated male rats

Rats that were left for week 10 – 12 recovery period showed significance differences between treated and ameliorated rats (Figure 5). Morphology of testes showed significant variation between treated groups. The circumference of testes of treated rats significantly increases at the 3rd week of treatment compared to weeks 3 – 6, 6 – 9 and during week 10 – 12 recovery period.

Testes length of control rats was constant at 6 weeks but reduced in length from the 9th week to the recovery period. Testes length of rats given Shea oil as abatement reduced at the 3rd week of treatment but increased from the 6th week to 9th week (Figure 5). There was also reduction in testes length during the recovery period. Rats treated with effluent alone without abatement showed no significant difference ($p>0.05$) in testes length during the recovery period.

Tissue Concentration of Heavy Metals: The testes were found to contain detectable concentrations of Chromium (Cr) and Lead (Pb). Tissue Cr and Pb concentrations were found to be significantly different ($p<0.05$) in rats treated with effluent only (Figure 6).

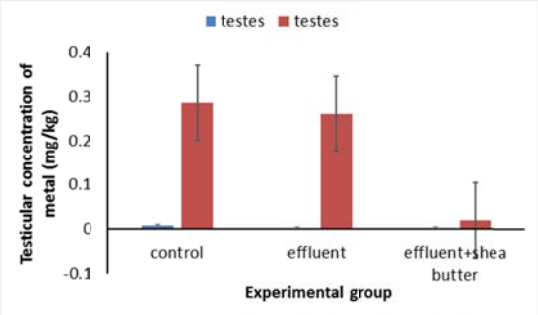

Figure 6: Tissue concentration of chromium (Cr) and lead (Pb) in the testes of control, effluent treated and Shea oil ameliorated male rats respectively

The maximum Cr and Pb concentration in gonads were in effluent treated rats. In the testes, the highest concentration of Cr (0.018 mg/kg) was observed at week 3 in the effluent treated rats. While the highest concentration of Pb (0.9 mg/kg) was observed at the recovery period.

Histopathology: The results of the histopathology of the testes of rats revealed that after 3 weeks of exposure the testis appeared normal in shape and morphology with no visible signs of degeneration composing of seminiferous tubules and interstitial space in the control group (Figure 7). Animals in Group 2 showed mild interstitial congestion and oedema (Figure 8). While the animals in Group 3 showed normal spermatogenic series (Figure 9).

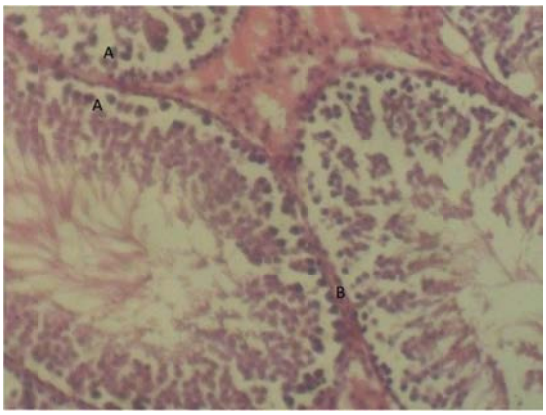

Figure 7: Testis of control rat showing (A) seminiferous tubules and (B) interstitial space (H & E x100)

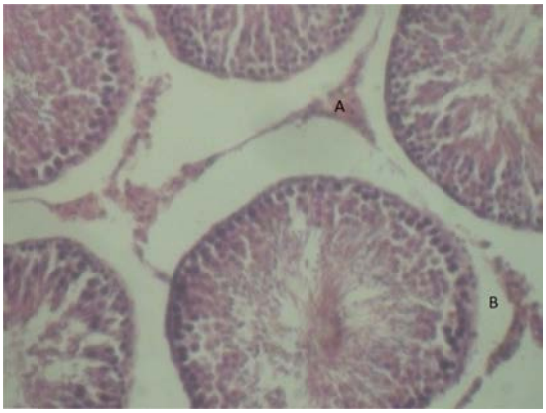

Figure 8: Testis of refinery effluent treated rat showing (A) mild interstitial congestion and (B) interstitial oedema (H & E x100)

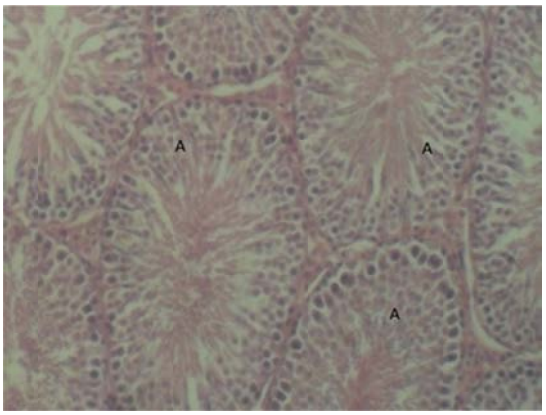

Figure 9: Testis of refinery effluent treated rat ameliorated with Shea oil showing (A) normal spermatogenic series (H & E x100)

At week 6, the testes of rats in the control group appeared normal with no visible signs of degeneration, composing of normal seminiferous tubules and normal interstitial space (Figure 10). The testes of rats given effluent only showing patchy spermatogenic arrest (Figure 11), while the testes of the rats given effluent and Shea oil showed normal tubules (Figure 12).

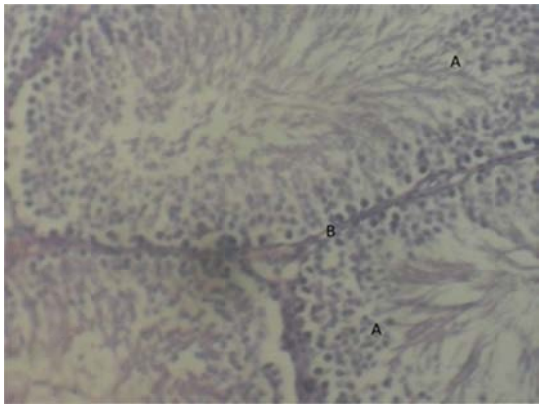

Figure 10: Testis of control rat showing (A) seminiferous tubules and (B) interstitial space (H & E x100)

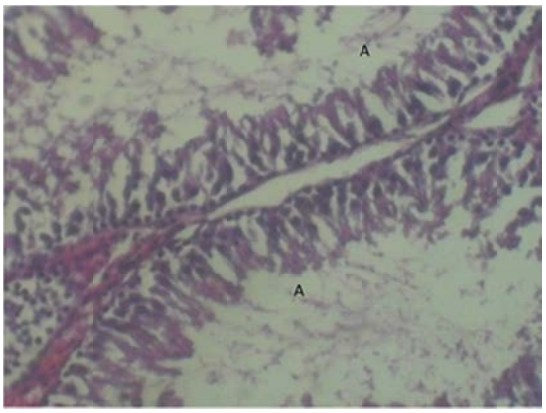

Figure 11: Testis of refinery effluent treated rat showing (A) patchy spermatogenic arrest (H & E x100)

At week 9, the testes of the rats given effluent only showed mild patchy spermatogenic arrest (Figure 13) while the testes of the rat given effluent ameliorated with Shea oil showed normal sequential maturation (Figure 14). At 21 days post exposure period the testes of the male rat in which effluent only was discontinued showed patchy spermatogenic arrest (Figure 15).

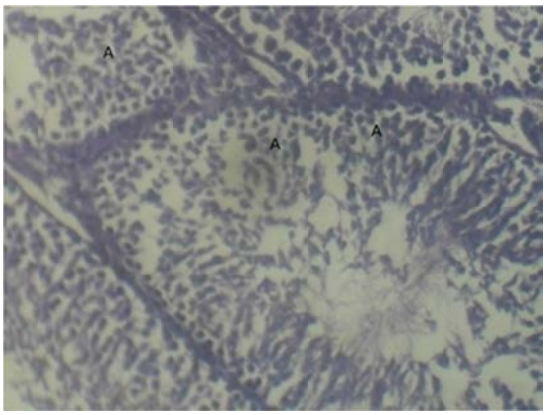

Figure 12: Testis of refinery effluent treated rat ameliorated with Shea oil showing (A) normal tubules (H & E x100)

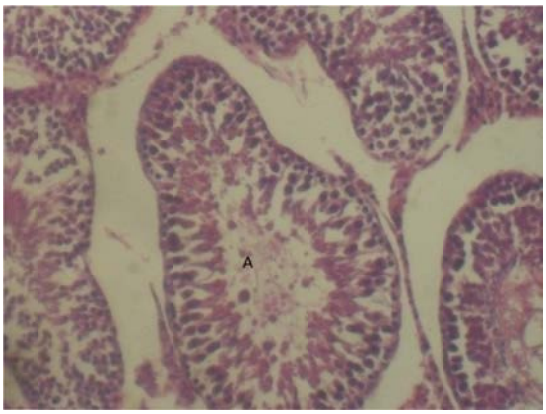

Figure 13: Testis of refinery effluent treated rat showing (A) patchy spermatogenic arrest (H & E x100)

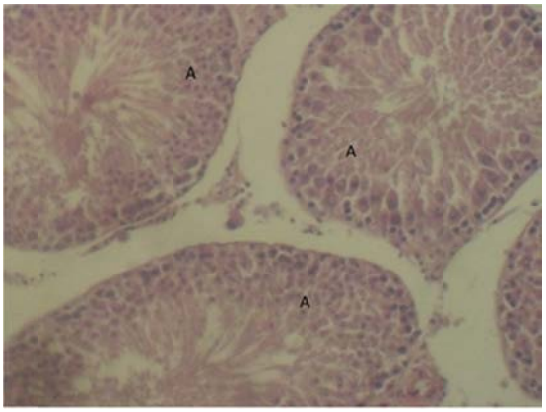

Figure 14: Testis of refinery effluent treated rat ameliorated with Shea oil showing (A) normal sequential maturation (H & E X100)

While the testes of the rats in which effluent and Shea oil was discontinued showed normal sequential maturation (Figure 16).

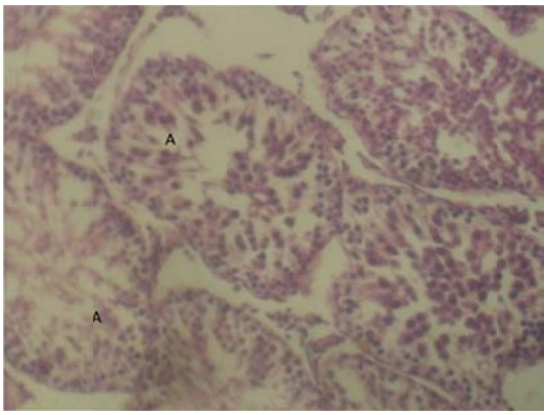

Figure 15: Testis of refinery effluent treated rat ameliorated with Shea oil after 21 days recovery period period showing (A) patchy spermatogenic arrest (H & E X100)

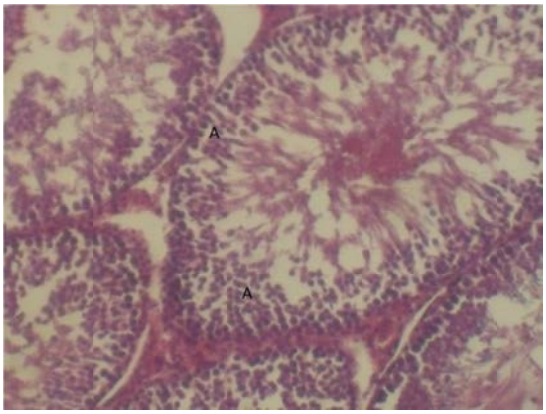

Figure 16: Testis of refinery effluent treated rat after 21 days recovery period showing (A) normal sequential maturation (H & E x100)

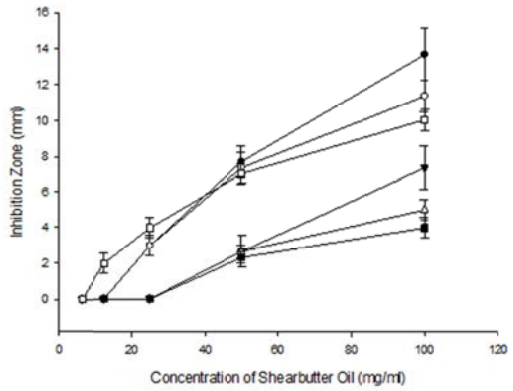

Figure 17: Antimicrobial inhibition of Shea oil on microbial culture of untreated refinery effluents at various concentrations

Antimicrobial Activities of Shea Oil: There was an increase anti-microbial activity with Shea oil (Figure 17). All the isolates were inhibited at concentration of 100 mg/ml of Shea oil with *Micrococcus* spp. and *Klebsiella* spp. having the highest and lowest zones of inhibition, respectively.

DISCUSSION

Shea oil extracted from Shea tree (*Vitellaria paradoxa*) is one of the essential oils, known to possess antimicrobial, antifungal, antiviral, insecticidal and antioxidant properties (Kordali *et al*., 2005). Shea oil is composed of five principal fatty acids: palmitic, stearic, oleic, linoleic and arachidonic acids. About 85 to 90 % of the fatty acid composition is stearic and oleic acids. Phenolic compounds are known to have antioxidant properties. A study characterized and quantified the most important phenolic compounds in Shea butter (Marouani *et al*., 2012). Shea oil used in this study demonstrated antimicrobial activities against all bacteria isolates from refinery effluent, antimicrobial activity by Shea oil against *Staphylococcus aureus* has seen reported earlier (Wahedi and David, 2014).

Results of this study showed that fifteen days exposure of adult male rats to refinery effluent induced reproductive toxicity. The relative weight of testes was reduced in effluent treated rats. Significant increase in body weight gain across the duration of exposure was seen in rats treated with refinery effluent and those given Shea oil for abatements. In the period when rats were withdrawn from treatment i.e. recovery period, there was significance reduction in body weight across the treated groups. Although, rats during exposure showed no obvious signs of loss of appetite as rats feed and consumed water that was added *ad-libitum*, the increased body weight might be an indication of toxicity via accumulation of fluids in organs or other body tissues and heavy metal accumulation in tissues and organs of treated rats. Studies have reported weight gain in some organs of rats upon exposure to contaminants such as leachates and the authors attributed it to the heavy metal constituents in the

contaminants this may be responsible for the change noticed in organ weight in this study as these organs are involved with sequestration of metals (Barbier *et al.*, 2005). A similar study reported reduction in body and organ weight of rats exposed to textile effluent at concentrations reported toxic (Suryavathi *et al.,* 2005). Similar to previously published reports, testicular Cr and Pb levels increased in a dose-dependent manner in treated rats, confirming the absorption of the metals after administration (Marouani *et al.,* 2012).

Moreover, histological changes observed in the gonads of treated animals, included enlarged intracellular space; which may have resulted from the destruction of Sertoli cells by the intoxication with heavy metals; as well as a dramatic loss of gametes in the lumen of the seminiferous tubule. This might also be a consequence of the disruption of the blood-testis barrier with the heavy metal accumulation in the tissues. Changes in the permeability of the blood-testis barrier and alterations in testicular and epididymal histo-architecture was also demonstrated in rats exposed to Cr (Marouani *et al*., 2012). Many reports exist on the effects of heavy metal accumulation in humans, but only a few studies have verified these effects in experimental animals (Flora *et al*., 2012). Oral exposure of both male and female rabbits to sodium dichromate (0.1% solution, 0.2 – 5 mg/kg body weight for up to 545 days) resulted in significant morphological changes in the gonads, including atrophy of the epithelium and dystrophic alterations of the Sertoli and Leydig cells in the testes (Nigam *et al*., 2014). Large doses of hexavalent Cr are highly toxic and may cause death especially when injected subcutaneously (Nigam *et al.,* 2014). A study in which sodium dichromate (VI) was administered by gastric intubations to groups of 10 mature male rats at levels of 20, 40 and 60 mg Cr(VI)/kg/day for 90 days also reported reproductive effects in rats and mice orally exposed to Cr(VI) (Chandra *et al.,* 2007).

In this study, the highest concentration of Cr in the testes (slightly > 0.018 mg/kg) was at 3 weeks in the effluent treated rats while for Pb, the highest concentration (0.9 mg/kg) was at the recovery period.

The testis of the rats in the refinery effluent treated and Shea oil ameliorated group showed mild inflammatory changes (interstitial congestion and oedema) similar to that seen in Pb intoxication; the ameliorated group did not show these pathologies at 3 weeks. There was some degree of spermatogenic arrest after six weeks in the effluent intoxicated group, which was indicated by alterations of the normal testicular architecture. There have been previous report of damage to testicular architecture in rabbits given a dose of 200 mg of Pb this study reported deformities in the architecture with serious damage within the seminiferous tubules (Ibrahim *et al.,* 2012). However, these pathologies were abated in the group treated with Shea oil in this study. Some degree of spermatogenic arrest also occurred at the 9th week, which abated in the Shea oil treated group also. At the end of the three weeks recovery period, the spermatogenic arrest reduced with visible signs of return of the testes to normal testicular architecture.

Shea oil had inhibitory action against the six bacteria isolates (*Staphylococcus aureus, Micrococcus, Bacillus subtilis, Klebsiella* spp., *Escherichia coli* and *Pseudomonas aeruginosa*) from the refinery effluent. This agrees with previous reports (Coulibaly *et al*., 2009; Wahedi and David, 2014). This study has provided adequate information on the toxic effects of untreated refinery effluent on the testes of rats. The histological examinations revealed varying pathological changes in the testis. These include spermatogenic arrest. However, the use of Shea oil for amelioration of the toxicity induced by the untreated refinery effluent proved effective.

Conclusion: The study concluded that; the level of heavy metal concentration on the testicles of the rats exposed to refinery effluent was high, there was histopathological alteration in the testes of the effluent treated rats, Shea oil proved effective against the toxic effects of the untreated refinery effluent on the testes of treated rats. Finally untreated refinery waste should be treated and disposed of properly to avoid contaminating the environment and

causing deleterious effects on organs and tissues of unsuspecting animals and possibly humans.

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