PROTECTIVE EFFECT OF SHEA OIL ON THE OVARY OF ALBINO RATS INTOXICATED WITH REFINERY EFFLUENTS

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ABSTRACT

This study reports the effect of untreated refinery effluents on female albino rats ameliorated with Shea oil. The experimental set-up included three treatment groups (effluent only, effluent abated with Shea oil and the control) of adult albino rats 6 – 7 weeks old. Rats were acclimatized for a period of 2 weeks before been orally administered 2 ml of untreated refinery effluent by oral gavage and were sacrificed at 3, 6 and 9 weeks interval. After 9 week, rats were left for 21 days post-exposure to recuperate. Body, organ weight and morphology, heavy metal concentration in ovaries and histopathology of ovaries were estimated across the groups. There was significant increase (p>0.05) in body and organ weight of treated rats compared to control. Organ morphology of treated rats varied from control but was not statistically significant (p>0.05). Lead and chromium concentrations in ovaries were high in concentration in rats treated with effluent only. Histopathology of ovaries showed evidence of pathological changes in rats treated with effluent when compared with control and Shea oil ameliorated group. Rats ameliorated with Shea oil showed early return to normal histological architecture in their ovaries. Shea oil was effective in ameliorating the effects of untreated refinery effluent toxicity on the ovary of intoxicated female albino rats.

Keywords: Shea oil, Abatement, Refinery effluents, Chromium, Lead, Toxicity, Ovary, Rats

INTRODUCTION

The effect of petroleum refinery effluents 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 effluents which contains heavy metals may occur through direct exposure to contaminated substrate such as soil, air, water and food or via consumption of aquatic lives via biomagnification (Marouani *et al.,* 2011). Heavy metals found in petroleum refinery effluents such as chromium, lead, mercury, copper and zinc have received special attention in ecotoxicology in recent years (Akunna *et al.,* 2012). 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 (Balk *et al.,* 2007).

In Nigeria, the exploration of crude oil and improper waste disposal after refining brings about the pollution of our environment including our waterways. Effluents such as textile dye effluents have been reported to cause several toxicological effects which include loss of body weight, reduction in organs, disruption of haematological profile and

histopathological alterations in organs (Sharma *et al.,* 2008).

The therapeutic role of medicinal plants in ameliorating the toxic effect of effluents and other contaminants has been widely reported (Chatterjee *et al.,* 2012; Ugwu *et al.,* 2013; Adikwu *et al.,* 2013). Essential oils such as Shea oil have also been utilized as therapeutic supplements since they are rich in biologically active compounds but there is paucity of information on their role in amelioration of toxicity resulting from environmental pollutants such as refinery effluents.

Shea oil which is extracted from Shea tree (*Vitellaria paradoxa*) is one of the essential oils which have been shown to possess antimicrobial, antifungal, antiviral, and insecticidal and antioxidant properties (Alander and Andersson, 2002; Maranz *et al.,* 2003; Akhter *et al.,* 2008; Garba and Salihu, 2011). Shea oil is composed of five fatty acids: palmitic, stearic, oleic, linoleic, and arachidonic acids. About 85 to 90 % of the fatty acid composition is stearic and oleic acids. A recent study characterized and quantified the most important phenolic compounds in Shea oil (Honfo *et al.,* 2014). Phenolic compounds are known to have antioxidant properties against tissue damage. The aims of this study therefore was to determine the level of heavy metal concentration in the ovary of albino rats intoxicated with untreated refinery effluents, observe the histopathological changes in the ovary, determine the ameliorative effects of Shea oil on the toxicity resulting from exposure to untreated refinery effluent and determine the recuperation time of rats post exposure.

MATERIALS AND METHODS

Experimental Design: A total of 30 female albino rats were used for the experiment. Ethical Clearance was obtained from the University of Benin, Committee on Animal Handling and Care before the commencement of the work. Rats were randomly assigned into 3 groups of 10 animals each. Rats in group 1 were given feed with drinking water *ad-libitum* all through the experiment. These served as the control group. Group 2 rats were given feed with drinking water *ad-libitum* and also received 2 ml of 100 % of the untreated refinery effluent continuously for 9 weeks. These served as the treatment group. Group 3 rats were given feed with drinking water *ad-libitum*, 2 ml of 100 % of untreated refinery effluent and 2 ml of Shea butter oil continuously for 9 weeks. This served as the ameliorated group. At 3 weeks intervals, two (2) rats were euthanized from each groups (1 – 3), blood and tissue samples were collected and sent to the laboratory for analysis till the end of the exposure phase. Treatment and amelioration was discontinued after nine weeks. The remaining rats in the treated and ameliorated groups 2 and 3 respectively were designated groups 4 and 5. They were untreated for 21 days but were given food and drinking water ad-libitum. After this post exposure phase, blood and tissue samples from groups 4 and 5 were collected and sent to the lab for analysis.

Physico-chemical Analysis of Refinery Effluent: The stock refinery effluent was collected from a crude oil refinery (including both the tank farm drainage water and the spent caustic and monoethanolamine (MEA); transferred to the laboratory in pre-cleaned 1.5 liter plastic containers and stored at room temperature until use. Physical and chemical components of the untreated refinery effluent were analyzed and parameters such as pH, temperature, bicarbonates, sulphates, phenols, total nitrogen, total suspended solids (TSS), turbidity and heavy metals constituents were determined using Flame Atomic Absorption Spectrophotometer (210 VGP Atomic Absorption Spectrophotometer, Buck Scientific, East Norwalk, USA) (Harvey, 2000).

Phytochemical Analysis of Shea Oil: Qualitative phytochemical tests were conducted to determine the levels of flavonoids, tannins, cardiac glycosides, saponins, steroids, terpenoids, alkaloids, and reducing sugar in the Shea oil (Harborne, 1973).

Anti-microbial Analysis of the Effluent and Microbial inhibition using Shea Oil: Untreated refinery effluents was analysed for

presence of microbes as described by Barrow and Feltham (1983). Shea oil was used to test for anti-microbial efficacy on isolated microorganisms from the effluents and zones of inhibitions were recorded (Barrow and Feltham, 1983) before the commencement of the experiments

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

Isolation, enumeration, characterization and identification of microorganisms: 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. Colony counts were taken and recorded in colony forming unit per milliliter (cfu/ml). The bacterial isolates were identified based on standard microbiological methods (Barrow and Feltham, 1983). Bacterial cultural characteristics and fungal isolates were identified based on their macroscopic and microscopic characteristics with reference to standard identification keys and atlas (Karabudak *et al.,* 2015).

Physical Observations, Body and Organ Weight Measurement: Rat in each of the groups were observed twice daily (before and after exposure) for signs of clinical toxicity in the skin and fur, eyes and mucous membrane, behavioral pattern, respiratory system, morbidity and mortality were also noted. The body weight of each animal in the control and treatment groups were measured at the beginning of the experiment and at the end of the exposure period using OHAUS Scout Pro Electronic Balance (Model: SPU202). At the end of the exposure period the ovaries of the animals were surgically removed, weighed and fixed with Bouins fluid for histology.

Heavy Metals Concentration Analyses: Heavy metal concentrations in the organs were determined by atomic absorption spectrophotometry (210 VGP Atomic Absorption Spectrophotometer, Buck Scientific, East Norwalk, USA) (Harvey, 2000).

Histopathology of ovary: The fixed ovaries were dehydrated in ascending concentrations of alcohol, cleared in xylene for 90 minutes, and embedded in paraffin wax. Sections 5 microns thick were made and mounted on slides. The slides were stained with Haematoxylin, counter stained with Eosin (H & E stains) and viewed under an Olympus Light Microscope (Nikon Eclipse E400) All alterations from the normal structure were registered. Photomicrographs were obtained at different magnification to show the histological changes in the ovarian tissues of rats form the experimental groups at varying exposure and durations.

Statistical Analysis: Collected data were analyzed using analysis of variance (ANOVA). Significant means were separated using Duncan Multiple Range test (Duncan, 1955). Means were adjudged significantly different at $p \leq 0.05$. All data were analyzed using the statistical software, SigmaPlot Version 12.0 for Windows.

RESULTS

Physico-chemical Characteristics of Refinery Effluent: Physico-chemical tests revealed that the 100 % concentrated effluent was generally colourless with putrid smell, slightly acidic (pH = 6.49), TSS (2.4 mg/l), sodium (26.9 mg/l), potassium (8.9 mg/l), calcium (5.6 mg/l), magnesium (5.6 mg/l), chloride (5.6 mg/l), sulphates (25.9 mg/l) and bicarbonates (3.1 mg/l) (Figure 1).

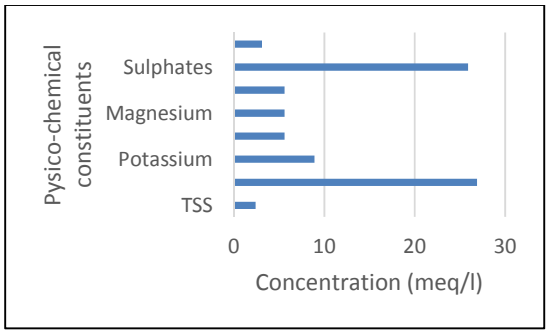

Figure 1: Physico-chemical constituents of refinery effluents

Phytochemicals in Shea Oil: Shea oil was found to be rich in flavonoids, terpenoids, steroids, anthragiones, saponin, reducing sugar, alkaloids, tannin and cardiac glycosides (Table 1). These essential constituent could serve as anti-oxidants and are also useful in nutrient enrichment.

Table 1: Phytochemical constituents of Shea oil

Phytochemical Constituents of Shea Oil	
Flavonoids	++
Terpenoids	++
Anthraquinones	++
Saponin	++
Alkaloids	++
Tannin	++
Reducing Sugar	++
Cardiac glycosides	++

++ Moderately present

Microbial Composition of the Effluent and Shea Oil Activity on Microorganisms: Microbial isolates of *Micrococcus* spp., *Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Klebsiella* spp. and *Staphylococcus aureus* were found in the refinery effluents (Table 2).

Table 2: Microbial constituents of effluents

Microorganisms	Inhibition by Shea oil
Micrococcus spp	++
Bacillus subtilis	++
Pseudomonas aeruginosa	++
Escherichia coli	++
Klebsiella spp	++
Staphylococcus aureus	++

++ Moderately present

Shea oil was found to inhibit growth of these microorganisms found in the effluents at varying concentrations (Figure 2).

Body Weight: There was a constant gain in body weight observed in rats treated with effluent and Shea oil (Figure 3). Table 4 revealed that there was significance difference ($p<0.05$) between mean weight gain in test groups and the initial weight at 1 – 3, 4 – 6 and 7 – 9 weeks respectively.

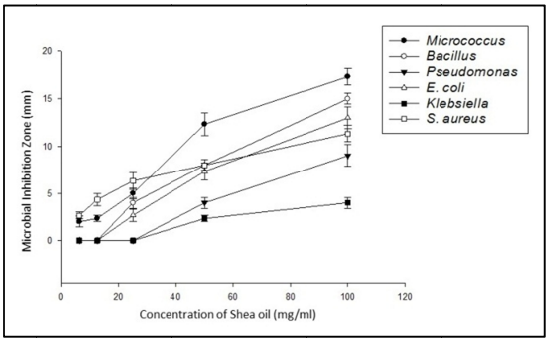

Figure 2: Inhibition of microbial growth from refinery effluents by Shea oil

However, average weight gain was observed to be only significantly different within the various time points at 1 – 3 weeks. The ANOVA revealed that the mean body weight differed significantly between 1 – 3 weeks ($p<0.0001$); 4 – 6 weeks ($P<0.0001$) and 7 – 9 weeks ($p<0.05$) respectively. At the recovery periods (10 – 13 weeks), mean difference in body weight was not significantly different ($p>0.05$). The least mean weight was observed amongst the Shea oil treated group (Table 3).

Ovary

Ovary weight: Ovary weight and morphology was not estimated because the ovaries of some rats in some groups had atrophied. Thus, there was no comparison between ovarian weights.

Ovarian concentration of heavy metals: The ovaries were found to contain certain detectable concentrations of chromium and lead. Chromium (Cr) and lead (Pb) concentrations were found to be significantly different ($p<0.001$) in the ovaries of rats treated with effluent only without amelioration with Shea oil. The maximum chromium and lead concentration were found in effluent treated rats. In the ovary, the maximum value of chromium (> 0.02 mg/kg) was detected at 3 weeks in the effluent treated rats while the maximum concentration of lead (0.9 mg/kg) was detected in the ovary of rats treated with effluent only at the post-treatment period (Figure 4).

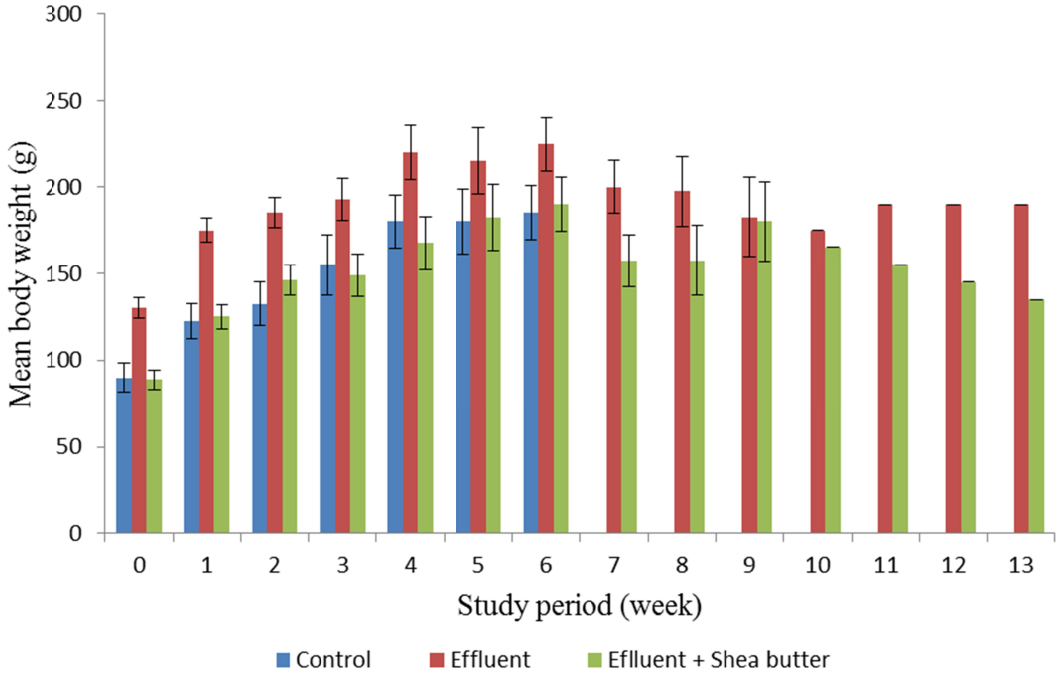

Figure 3: Changes in mean in mean body weight of female subjects across experimental and control groups respectively during and after amelioration

Table 3: Results of repeated measures ANOVA and test of significance of mean body weights between various time points across the various experimental groups

Time period (week)	Time point	Mean body weight (g)	SE	95% CI		Compared time points	P
				From	To		
1 - 3	0	102.917	3.921	93.644	112.189	0 - 1	<0.0001
	1	140.833	4.735	129.637	152.030	0 - 2	<0.0001
	2	154.583	5.884	140.67	168.497	0 - 3	<0.0001
	3	165.417	8.159	146.124	184.709	1 - 2	0.003
4 - 6	0	98.333	5.415	83.299	113.367	0 - 4	0.014
	4	173.333	12.454	138.757	207.91	0 - 5	0.001
	5	173.333	6.395	155.578	191.088	0 - 6	<0.0001
	6	182.778	1.757	177.9	187.655	4 - 5	>0.05
7 - 9	0	106.25	8.004	71.812	140.688	0 - 7	0.101
	7	178.75	10.68	132.798	224.702	0 - 8	0.067
	8	177.5	14.252	116.178	238.822	0 - 9	0.104
	9	181.25	16.25	111.332	251.168	7 - 8	>0.05
10 - 13	10	170.54	6.954	159.456	193.345	10-13	>0.05
	11	172.58	9.455	87.23	121.678	10-13	>0.05
	12	167.53	8.225	141.267	176.982	10-13	>0.05
	13	162.57	13.37	143.87	201.354	10-13	>0.05

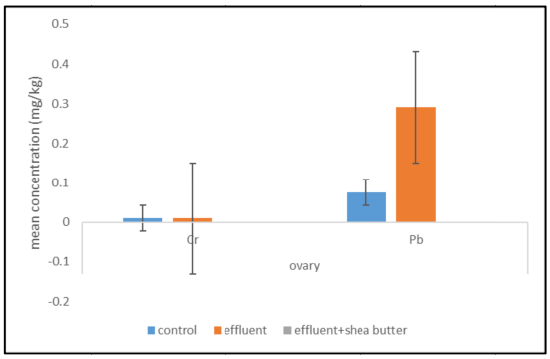

Figure 4: Mean concentration of chromium (Cr) and lead (Pb) in the ovary of albino rats

Histopathology of the ovary: The ovary of the rat in which effluent only was given showed enucleated follicle and decidualized stroma (Figure 5 a).The ovary of the rat in which effluent and Shea oil was given showed follicles in different stages of development (Figure 5 b). The ovary of the rat in which effluent and Shea oil was discontinued post exposure period showed ovarian follicles in different stages of development (Figure 5 c).

DISCUSSION

The weight and morphology of the ovaries was not estimated in this study because the ovaries of some of the rats were atrophied. However, previous studies have reported increase in ovary weight in rats exposed to Escravos crude oil (Okoye *et al.,* 2014). The observed increase in ovarian weight has been attributed to enlargement of follicles in the ovary which is probably induced by the crude oil. The urinalysis of rats after 6 weeks of treatment in this study showed normal with pH being neutral as against the control which was tending towards alkalinity. The ovarian atrophy recorded in this study was similar to earlier studies in which the uterus of chromium-treated rats showed atrophy of endometrial glands, fibrous tissue proliferation and hyperplasia of uterine epithelium. In this study, ovarian sections from group 2 revealed severe congestion and degenerated follicles (Balakrishnan *et al.,* 2013). In addition, cystic follicles were seen in large numbers. Ultra-structural changes like distorted nucleus, swollen and elongated mitochondria, altered epithelial size and shape were also noticed in effluent treated rats. It has been reported that many environmental toxicants gain access to the ovary via blood circulation (Elbetieha and Al-hamood, 1997). The risk of damage of the ovarian follicle cell population from the toxicants depends mainly on the accessibility of toxicants to ovarian follicles. Previous study found out that there was a decrease in uterine weight and lipid peroxidation of female mice treated with chromium trichloride (Elbetieha and Al-hamood, 1997). A decrease in the percentage of vaginal opening time in female rats treated with potassium dichromate as an index of delay onset of puberty in female rats and decreased in number of primordial, primary and secondary follicles with no observation of antral follicles has also been shown in an earlier study (Banu *et al.,* 2008). In addition, translactational rats exposed to potassium dichromate (VI) showed reduction in the number of ovarian follicles, steroidogenic ability, delayed puberty and increased interval between estrous cycles, which is in agreement with findings in this study with reduced diameter of ovarian follicles seen in atrophied ovaries. In this study, significant increase in body weight gain across the durations of exposure was observed in rats treated with refinery effluent and also those given Shea 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 observed. Though, it has been reported that rats exposed to industrial effluents do reduce in their body weight after exposure but this study found an increase in body weight of rats upon treatment. Although, rats during exposure showed no obvious signs of loss of appetite as rats fed 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 as well. However, contrary study has shown reduction in body and organ weight of rats exposed to textile effluent at toxic concentrations (Suryavathi *et al.,* 2005). This study also reported an increase in organ weight of treated rats with reduction in morphology such as length this was attributed

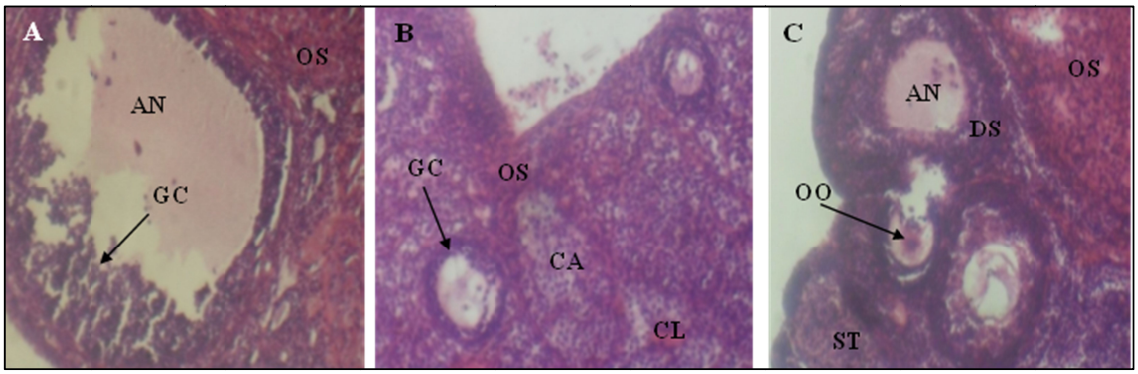

Figure 5: Showing mature ovaries of rat treated with effluent only at 9 weeks in (A) and effluent + Shea oil treated at 9 weeks in (B) and 13 weeks (C). Note the antrial fluid (AN), the granulosa cells (GC), the ovarian stroma (OS) in (A) is compromised. Note in (B) the corpus albican (CA) and corpus luteum (CL). In (C), note the intact integrity of the ovarian stroma and the oocyte (OO) in ruptured Graffian follicle (H&E x 100)

to accumulation of heavy metals in the affected tissues and organs. Previous studies have reported weight gain in some organs of rats upon exposure to contaminants such as leachates and the authors attributed it to heavy metal constituents in the contaminants and sequestration of metals in organs and tissue (Feuston *et al.,* 1997; Barbier *et al.,* 2005; Ogara *et al.,* 2016). In the ovary, the maximum value of chromium (>0.02 mg/kg) was detected at 3 weeks. Chromium was also high in ovary of treated rats (>0.01 mg/kg) at the 6^{th} week of treatment. The maximum concentration of lead (0.9 mg/kg) was detected in the ovary of rats treated with effluent only at the post-treatment period. After 9 weeks of exposure, rats in the effluent treated groups showed some level of degenerated follicular architecture with follicles not having ovum (enucleated). This agreed with the previous report of Balakrishnan *et al.* (2013). While others had disorganized granulose cells and stromal inflammation, the Shea oil treated group showed follicles in the different stages of development (maturation), showing recovery from the damaged state. Essential oils have been shown to possess antibacterial and antioxidant properties (Honfo *et al.,* 2014) due to the presence of some biologically active compounds in them. In this study, the essential oil used as abatement had antimicrobial activity against the bacteria isolated from the effluent. Shea oil had inhibitory action against the six bacteria isolates (*Staphylococcus aureus, Micrococcus* spp., *Bacillus subtilis, Klebsiella* spp., *Escherichia coli* and *Pseudomonas aeruginosa*) from the refinery effluent. Shea oil has been reported to have anti-microbial activity on *Escherichia coli* and *Staphylococcus aureus* (Wahedi and David, 2014). Histologically, untreated refinery effluent induced varying pathological changes in the ovary of adult female albino rats. These include follicular inflammation, ovarian degeneration and atrophy. However, abatement with Shea oil proved effective and potent. During the 21 days post exposure period ovarian follicles in the effluent treated group remained fairly damaged, the damage was not reversed, while the Shea oil treated group showed marked regeneration and improved ovarian function.

Conclusion: The study concluded that exposure to refinery effluents increased the level of heavy metal concentration in the ovary of albino rats and resulted to ovarian follicle degeneration and atrophy. However, ameliorating of exposure to untreated refinery effluents with Shea oil effectively reduced the level of ovarian tissue degeneration and atrophy and improved the rate of post exposure regeneration of the ovaries in exposed rats. Shea oil effectively ameliorated the toxic effect of refinery effluents on the ovaries of intoxicated albino rats.

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