MEDIRES
Symbol of Quality Research

Pollution and Effects on Community Health
info@mediresonline.org

ISSN: 2836-3590 RESEARCH ARTICLE

Investigation of the behavioral and neurotoxic effects of sub-chronic exposure of Wistar rats to treated crude oil exploration water (produced water)

Bakre A. G[1,2*]., Layemo K. A[1]., Olayemi J. O[3] & Vikosen E. N[4]

[1] *Department of Pharmacology and Therapeutics, College of Medicine, University of Ibadan, Nigeria.*
[2] *Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Ibadan, Nigeria.*
[3] *Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Nigeria.*
[4] *Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, Niger Delta University, Nigeria*

Corresponding Author:* Bakre A. G, Department of Pharmacology and Therapeutics, College of Medicine, University of Ibadan, Nigeria.

Received: 13 March 2023; **Accepted:** 18 April 2023; **Published:** 19 April 2023.

Citation: Bakre A. G (2023), Investigation of the behavioral and neurotoxic effects of sub-chronic exposure of Wistar rats to treated crude oil exploration water (produced water), Pollution and Effects on Community Health 2(2). DOI: 10.58489/2836-3590/015.

Abstract

Treated crude oil exploration water (TCOEW) is a significant byproduct created from petroleum exploration activities and is known to be a complicated synthesis of numerous dangerous chemical materials. This study is designed to evaluate the behavioral and neurotoxic effects of sub-chronic exposure of Wistar rats to treated crude oil exploration water (produced water). Fifty rats were randomly assigned to five treatment groups, with ten rats per group, and treated with five concentrations (1%, 5%, 10% and 20%) of TCOEW. Each TCOEW concentration was administered for 90 days *ad-libitum* as normal drinking water to each group, while the control group was given tap water. After sub-chronic exposure of 90 days, the behavioral effect of TCOEW was investigated using open field test (novelty-induced behavior), elevated plus maze (anxiety), novelty object recognition test (short-term memory) Morris water maze (spatial memory), and the neurotoxic effects by biochemical and histological assessment of the brain. Data were analysed using descriptive statistics and ANOVA at α 0.05. TCOEW produced a significant ($p < 0.05$) reduction in novelty-induced rearing, grooming, and locomotion. Also, TCOEW reduced the index of open arm entry IOAA in EPM, and time spent exploring the novel object in NORT. TCOEW-exposed groups had significantly higher annulus time and higher annulus crossing in Morri's water maze. This study provides evidence that the treated crude oil exploration water might contain chemicals, which on chronic exposure, have effect on behavior, learning and memory and anxiety.

Keywords: *Treated crude oil exploration water; novelty object recognition test; Chronic exploratory discharge; polycyclic hydrocarbons, phenols*

Introduction

Chronic exploratory discharge of *produced water* and drilling cuttings from oil and gas production into the water bodies in the Niger Delta regions has become a major source of pollutants; causing some health challenges and increasing the health risks for rural communities that depend solely on these natural sources for drinking, sustenance, and livelihood (Gazali *et al.,* 2017). Behavioral alterations and neurotoxic effect associated with exposure to petrochemicals has been reported by quite a number of researchers. It is an important component of environmental and occupational safety programs of humans exposed to these industrial compounds, which have proven toxic to the nervous system. Identification of the behavioral and neurotoxic syndromes attributable to exposure to produced water will help improve treatment of waste water before discharge and legislation on waste water disposal. Produced water is a complicated synthesis of numerous dangerous chemicals containing heavy metals, inorganic and organic compounds, and naturally occurring radioactive materials (NORMs) in

enormous proportion (Clinton 2009; Andrade *et al.* 2010). The two main disposal methods for produced water are ecologically threatening (Tellez *et al*. 2002). The chemicals contained in petroleum waste include large amount of aromatic and polycyclic hydrocarbons, phenols, sulphides, naphthylenics acids and the heavy metals derivatives of streams of compounds. Polyacramide-containing preparations cumulatively exhibit neurotoxicity characterized by anxiety, weakness, and cognitive impairment (LoPachin and Gavin, 2012). The empirical behavioral alterations associated with this neurotoxicity are explained by cerebellar Purkinje cell death and degeneration of distal axons and nerve terminals in the peripheral and central nervous systems (LoPachin 2004). There are experimental evidence of neurodevelopmental toxicity and reproductive toxicity (prenatal and perinatal exposure) due to petrochemicals (Garey and Paule 2010; Takahashi *et al.* 2009). However, there is little evidence that these neurotoxic consequences have human relevance (Haber *et al.*, 2009).

Standard behavioral tests are often used to verify theory of psychopathology. These animal models of human behavior represent a complex of cognitive and emotional processes in animals (particularly murine) that are translatable to human subjects (Belovicova *et al.,* 2017). Several of the behavioral tests measure motivation to perform task and spontaneous reactions of rodent in novel environment. The open field test (OFT) created in 1934 by Calvin S. Hall measures rodent emotionality. It is a standardized test which measure thigmotaxis (important indicator of anxiety) and habituation (exploratory behaviour) amongst other parameters. In a novel environment, excitement, emotionality and stress associated behavior are the primary reactions before animal habituates. Elevated plus maze also measures anxiety induced in rodent by neuroactive substances. Substances that increase time spent in the open arms by animal, are anxiolytics (Carola *et al.,* 2002). These overall effects are due to a resultant homeostatic and neuronal change in the brain.

The purpose of this study was to identify behavioral pattern exhibited by rats exposed to produced water for 90 days in anxiety (elevated plus maze), on memory (Morris Water Maze), and sought to explain via the biochemistry (GSH, SOD, Catalase) and histo-morphology. This model presents a set of data that could be extrapolated to human and might ensure proper regulation of waste water treatment process and disposal into water bodies.

Materials and Method

Collection and storage of produced water

The produced water herein called TREATED CRUDE OIL EXPLORATION WATER (TCOEW) was collected from the treatment plant of a crude oil exploring company in the Niger Delta region of Nigeria. It was transported to the Department of Pharmacology and Therapeutics, University of Ibadan, in plastic containers, where it was stored between 2 – 8 ˚C in a refrigerator.

Experimental Animals

Male Wistar rats weighing between 120 – 150 g were obtained from the central Animal House (CAH), College of Medicine, University of Ibadan. The animals were kept in plastic cages (42 x 30 x 27 cm) and fed with standard rodent pellet feed and water *ad libitum*. They were acclimatized for 1 week prior to the commencement of the experiments. Fifty animals were randomly distributed into five groups (n=10) according to the experimental design. Group 1 served as control (water), while the remaining groups 2, 3, 4 and 5 were the treatment groups receiving 1, 5, 10 and 20 produced water. The produced water were served with bottles (750ml) fitted with metal snout attached to each cage which were labeled control, TCOEW 2, TCOEW 3, TCOEW 4, and TCOEW 5, respectively. All methods in this study were carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). Ethical approval was obtained from the Animal Care and Use Research Committee, University of Ibadan.

Subchronic toxicity studies

The OECD guideline (OECD 407) for sub-chronic toxicity was adhered to. The animals were provided TCOEW as drinking water *ad libitum* at 1%, 5%, 10%, and 20%) for 90 days. For the control group, (0% v/v) tap water was administered. Animals were observed daily for general conditions; body weights were recorded once every ten days during the 90 days administration period. At the end of administration, behavioral tests were carried out to assess memory and locomotory effects. Also, the whole brain was dissected for estimation of markers of oxidative stress (malondialdehyde (MDA), superoxide dismutase (SOD), catalase, nitrite, and glutathione.

Behaivioural tests

Open Field Test

Open field box (72 × 72 × 36 cm) with one clear Plexiglas wall to allow visibility was used. The floor was divided into sixteen 18 x 18 cm squares and centre square (18 cm x 18 cm) in the middle (Brown,

et al., 1999). The open field maze was disinfected between each rat exploration using 70% ethyl alcohol. Rats were placed in the centre of the open field and allowed to explore the apparatus for 30 minutes. The behaviors scored include rearing, grooming, and line crossing (Brown *et al.,* 1999).

Elevated Plus Maze test: Anxiety test

The maze is elevated to a height of 55 cm with two open (50 × 10 cm) and two enclosed arms (50 × I0 × 55 cm), arranged such that the arms of the same type is opposite each other and connected by an open central area (I0 × 10 cm). The two enclosed arms will be darkened by two covers (50 × I0 cm). The rats were placed individually in the centre of the maze and observed for 5 min. The number of entries and time spent in open arms and closed arms were recorded. Arm entries were defined as entry of all four limbs into an arm of the maze. The maze was thoroughly cleaned with 70% alcohol after the removal of each rat.

Morris Water Maze: learning and memory test

Morris water maze is used to assess the impact of exposure to TCOEW on learning and spatial memory. The apparatus is a round (120 ×30 cm) cylindrical and opaque tank containing water which is made non-transparent (with milk) and a hidden (1 cm below water level) circular (12 cm) escape platform. The test is based on the ability of the rats to learn the location of the escape platform using contextual and visual cues in its spatial memory. The test is based on the principle that a rodent must escape by the fastest and most direct route from a seemingly dangerous water environment (Vorhees and Williams, 2014). The tank is divided into four quadrants marked north, south, west and east with the escape platform hidden in a particular spot in a specific quadrant. Each animal is dropped in the quadrant opposite the quadrant with the hidden escape platform, and allowed to locate it within 60 sec. If the animal is not able to locate the escape platform in 60 sec, it is carefully guided to the escape platform and allowed to stay on it for 1 min. All the animals are allowed to go through 4 trials (learning) per day for 3 consecutive days. The animals do not go through trial on the fourth day, but on the fifth day which is referred to as probe day the animals are allowed a single probe trial without the hidden platform. On the probe day, the single trial allow the animal to use its spatial memory to locate the quadrant that initially housed the escape platform. During the trials, the duration of time spent to locate the escape platform are recorded, while on the probe day the time spent in the target quadrant and the number of times the animal crosses the escape island are also recorded.

Object recognition test

The object recognition task was carried out in a square wooden open-field apparatus (60×60×40 cm) placed in a spatial room. The open field and the objects were cleaned between each trial using 70% ethyl alcohol to avoid odour trails. Objects of the same shape but different colors were used (Reppa *et al.,* 2020). Trial (test trial T1) was done by placing each animal in the arena containing two identical objects for 5 min allowing exploration of these two objects. Animals that do not explore the object for at least 15 sec during the 5 min test trials are excluded from the experiment. The animal explores the objects by sniffing, touching, or making close contact looking (interaction) at the object. On the test day, one similar object is replaced with a novel object (similar shape, but different colour), and the duration of time spent exploring the novel object within 5 min is recorded.

Brain Biochemistry

Rats were sacrificed through cervical dislocation immediately after the behavioral analysis. The whole brains were removed, weighed, and kept in the refrigerator. Afterward, the whole brain of each rat was homogenized with 5 mL of 10% w/v phosphate buffer (0.1M, pH7.4). Each brain tissue homogenate was centrifuged at 10,000 rpm for 10 min at 4 ˚C, the pellet was discarded, and the supernatant was immediately separated into different portions for different biochemical assays, namely: Superoxide Dismutase (SOD), Catalase (CAT), Reduced glutathione (GSH), Lipid peroxidation (Malondialdehyde, MDA) and Nitrite which were done according to their various principles, and methods described by Misra and Fridovich, (1972); Sinha, (1971); Jollow *et al.,* (1974) and Green *et al*., (1982) respectively.

Brain Histo-morphology

Rats were sacrificed through cervical dislocation immediately after the behavioral analysis, and the whole brains were removed and fixed in 10% buffered formalin. The fixed tissue were longitudinally dissected and placed in embedding cassettes before cutting into 4 µm section. Sections were then stained on slides using hematoxylin and eosin before observation under light microscope to obtain the histological photomicrographs captured using Olympus binocular microscope connected to a digital camera (Leica ICC50 E) and computer.

Statistical Analysis

The data were expressed as Mean ± S.E.M. (standard error of mean). The data was analyzed using Kruskal–Wallis test (Non-parametric) and one–way analysis of variance (ANOVA) followed by post–hoc test (Dunnet's test) for multiple comparisons where appropriate using Graph Pad Prism software version 5. A level of $p < 0.05$ was considered as statistically significant for all tests.

Results

Effect of TCOEW on Rearing and Grooming Behavior of Wister Rats

Administration of TCOEW caused a statistically significant (p <0.05) decrease in rearing and grooming in TCOEW-treated rats when compared to the control group (H_2O), as shown in Figure 1 below.

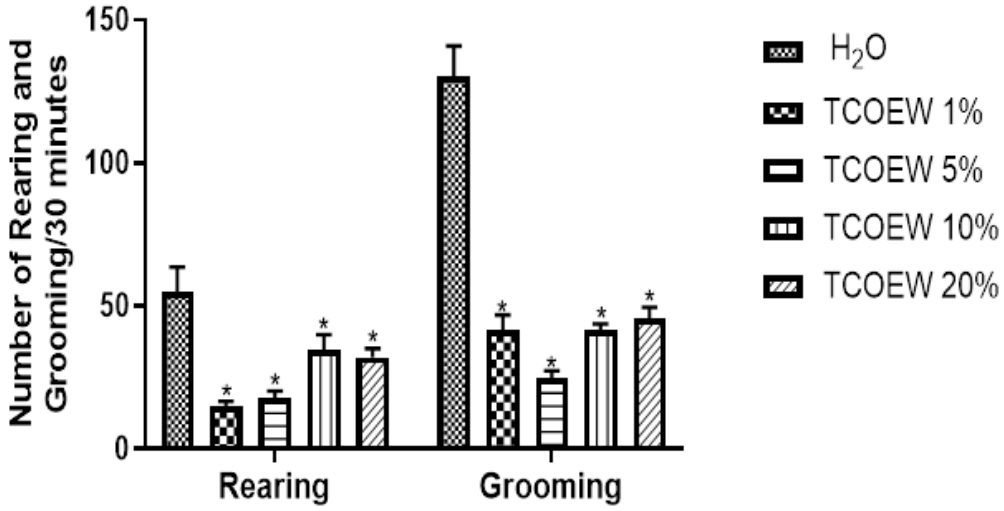


Fig 1: Effect of TCOEW on Rearing and Grooming Behavior of Wister Rats

All values were expressed as mean ± SEM (n = 6). Data were analysed using one-way ANOVA followed by Dunnet's *post-hoc* test. * = significance at $p < 0.05$ when compared with H_2O only. H_2O = Tap water, TCOEW = Treated Crude Oil Exploration Water

Effect of TCOEW on Wister Rat Locomotion in Open Field Test

When compared to the control group (H_2O), TCOEW 1%, TCOEW 5% group had a reduced locomotion which was statistically significant while TCOEW 20% increased locomotion was statistically significant and TCOEW10% decrease was statistically insignificant as shown in Figure 2 below

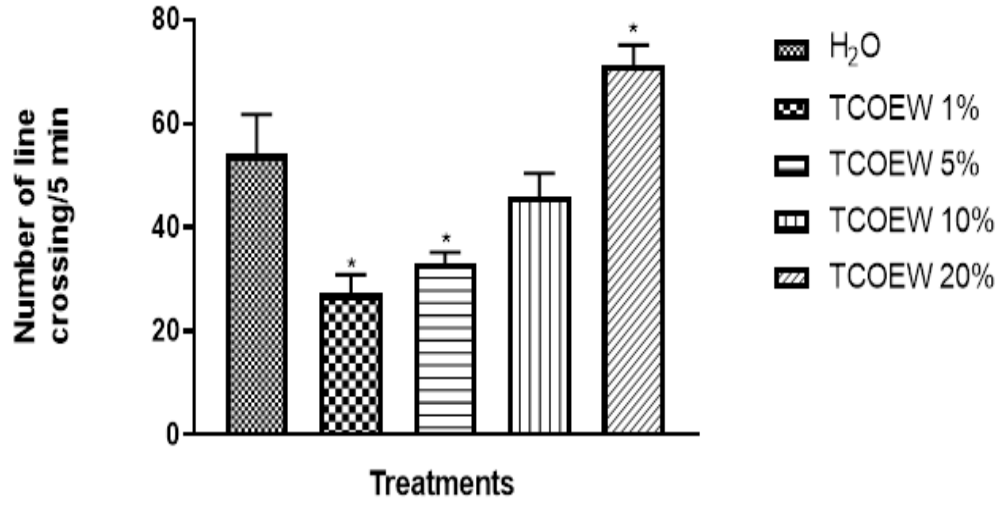


Figure 2: Effect of TCOEW on Wister Rat Locomotion in Open Field Test

All values were expressed as mean ± SEM (n = 6). Data were analysed using one-way ANOVA followed by Dunnet's *post-hoc* test. * = significance at $p < 0.05$ when compared with H_2O only. H_2O = Tap water, TCOEW = Treated Crude Oil Exploration Water.

Effect of TCOEW on Time spent in Open Arms and Closed Arms of Elevated Plus Maze (EPM) in Wister Rats.

When compared to the control group (H_2O), all the TCOEW groups had statistically insignificant (p >0.05) changes in the time spent in open arms and the time spent in closed arms as shown in Figure 3 below

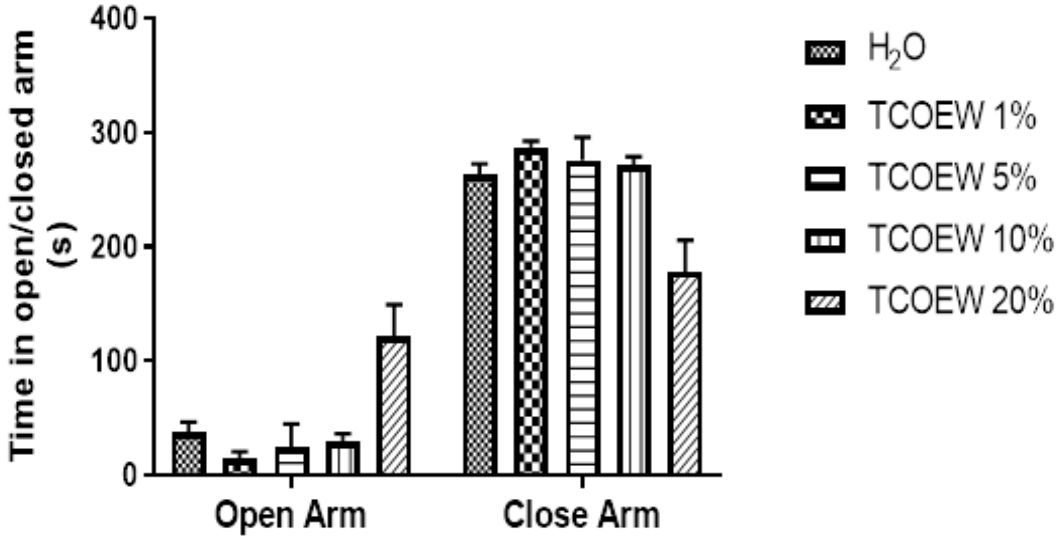


Fig 3: Effect of TCOEW on Time spent in Open Arms of Elevated Plus Maze (EPM) in Wister Rats.

All values were expressed as mean ± SEM (n = 6). Data were analysed using one-way ANOVA followed by Dunnet's *post-hoc* test. H_2O = Tap water, TCOEW = Treated Crude Oil Exploration Water

Effect of TCOEW on Number of Entries into Open Arms and closed Arms of Elevated Plus Maze (EPM) in Wister Rats.

Exposure to 1%, 5% and 10% showed increase in number of entries into open arm, but only 5% was statistically significant. In the close arm groups 2, 5 and 6 (TCOEW 1%, 20%) showed a statistically significant reduction in number of entries per 5 minutes into closed arms when compared with the control group (H_2O) as shown in Figure 4 below.

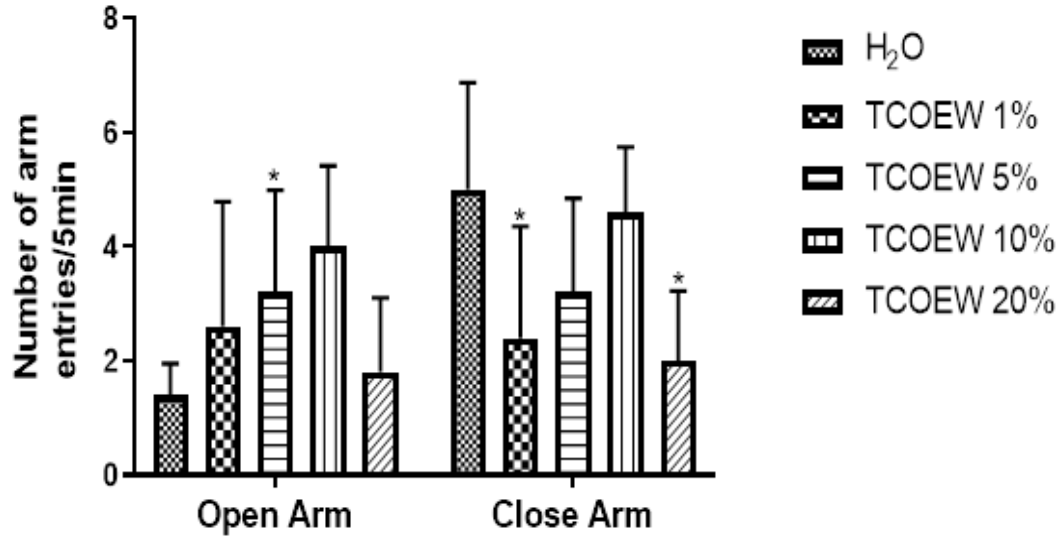


Fig 4: Effect of TCOEW on Number of Entries into Open Arms and closed Arms of Elevated Plus Maze (EPM) in Wister Rats.

All values were expressed as mean ± SEM (n = 6). Data were analysed using one-way ANOVA followed by Dunnet's *post-hoc* test. * = significance at $p < 0.05$ when compared with H_2O only. H_2O = Tap water, TCOEW = Treated Crude Oil Exploration Water

Effect of sub-chronic TCOEW exposure on Index of Open Arm Avoidance (IOAA) of Elevated Plus maze in Wister rats

The TCOEW treated groups showed varying

reductions in the Index of open arm avoidance (IOAA%) when compared with the control group (H_2O); TCOEW 5% and TCOEW 20% groups all showed statistically significant (p <0.05) reduction in index of open arm avoidance, while TCOEW 1% and TCOEW 10% showed statistically insignificant decrease in index of open arm avoidance.

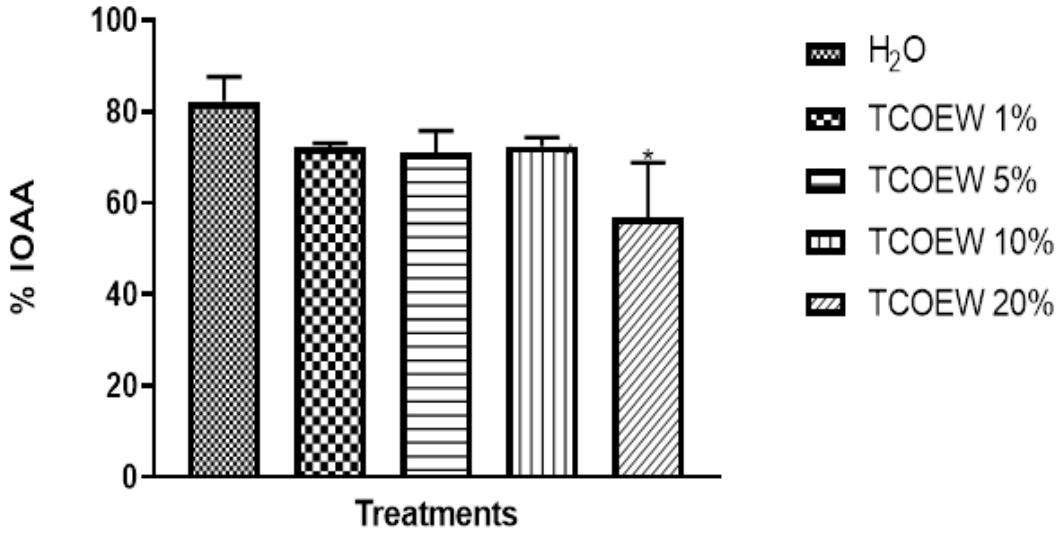
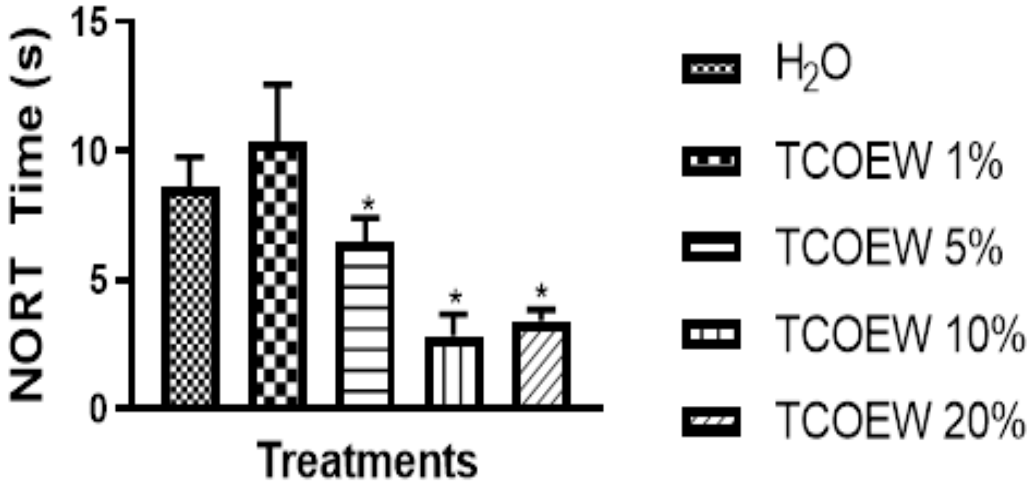


Fig 5: Effect of sub-chronic TCOEW exposure on Index of Open Arm Avoidance (IOAA) of Elevated PluWister rats

All values were expressed as mean ± SEM (n = 6). Data were analysed using one-way ANOVA followed by Dunnet's *post-hoc* test. * = significance at p < 0.05 when compared with H_2O only. H_2O = Tap water, TCOEW = Treated Crude Oil Exploration Water

Effect of TCOEW on Novel Object recognition test (NORT) in Wister rats

The TCOEW treated groups showed reduction in the Time spent exploring the novel object when compared with the control group (H_2O); TCOEW5%, TCOEW10% and TCOEW20% group all showed statistically significant (p <0.05) reduction Time spent exploring the novel object as shown in Figure 6 below.



.

Fig 6: Effect of TCOEW on Novel Object recognition test (NORT) in Wister rats

All values were expressed as mean ± SEM (n = 6). Data were analysed using one-way ANOVA followed by Dunnet's post-hoc test

* = significance at p < 0.05 when compared with H_2O only. H_2O = Tap water,TCOEW = Treated Crude Oil Exploration Water

Effect of TCOEW on Escape Latency of Morris Water Maze (MWM) in Wister rats

The escape latency of all the groups reduced but the most important was the reduction in the latency time

of TCOEW1% treated group in Trial 2 as shown in Figure 7 below.

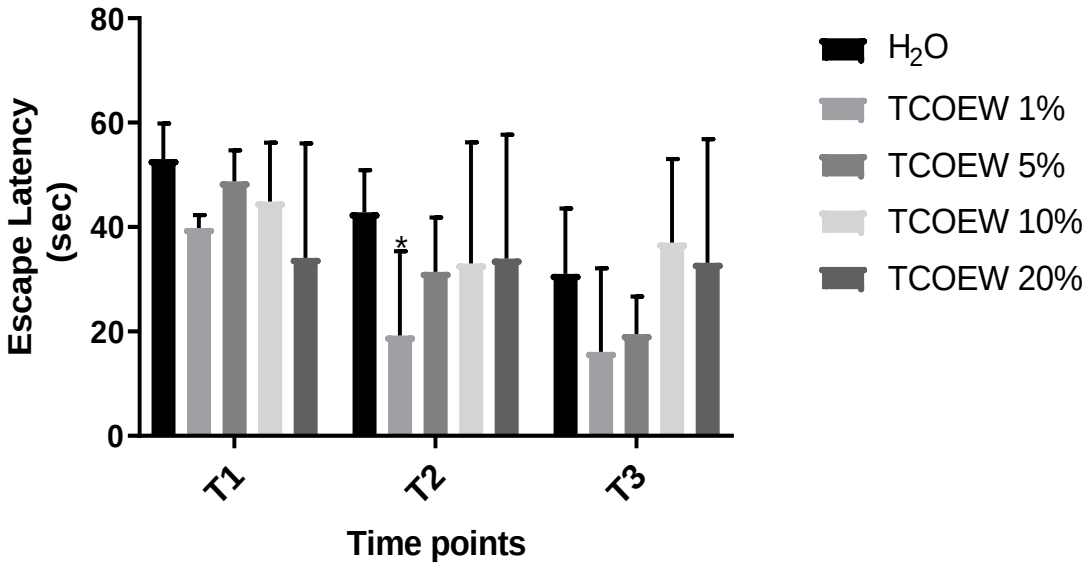
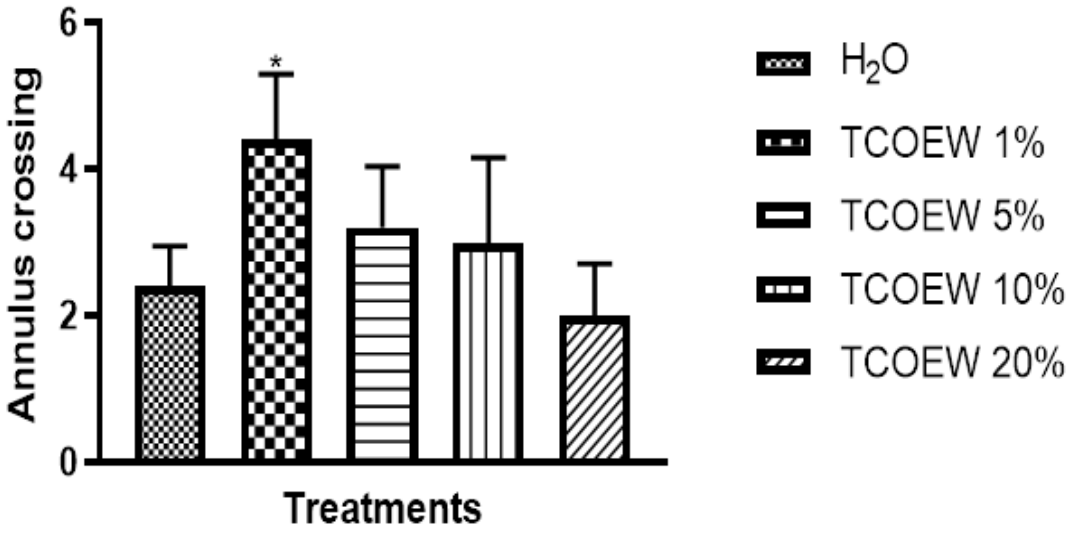


Fig 7: Effect of TCOEW on Escape Latency of Morris Water Maze (MWM) in Wister rats

All values were expressed as mean ± SEM (n = 6). Data were analysed using one-way ANOVA followed by Dunnet's *post-hoc* test. * = significance at $p < 0.05$ when compared with H_2O only. H_2O = Tap water, TCOEW = Treated Crude Oil Exploration Water

Effect of TCOEW on Annulus Crossing in Morris Water maze in Wister Rats

TCOEW initially caused a significant increase in annulus crossing followed by other insignificant increase and a little decrease in TCOEW20% group as shown in Figure 8 below.



.

Fig 8: Effect of TCOEW on Annulus Crossing in Morris Water maze in Wister Rats

All values were expressed as mean ± SEM (n = 6). Data were analysed using one-way ANOVA followed by Dunnet's *post-hoc* test

* = significance at $p < 0.05$ when compared with H_2O only. H_2O = Tap water, TCOEW = Treated Crude Oil Exploration Water

Effect of TCOEW on Annulus Time in Morris Water maze in Wister Rats

All TCOEW treated groups 1% - 20% showed a statistically significant (p <0.05) increase in the annulus time as shown in figure 9 below when compared with the control group (H_2O).

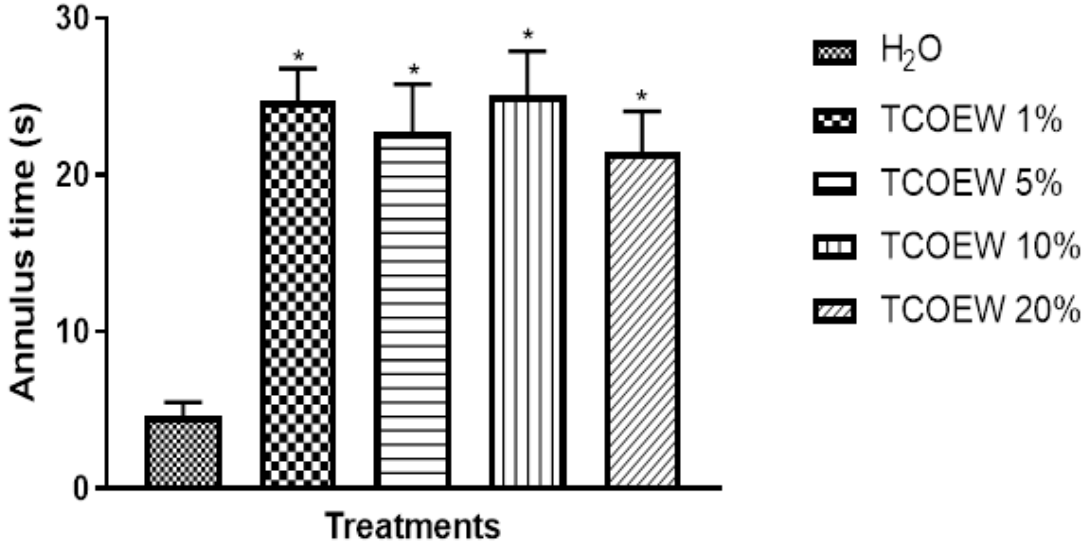


Fig 9: Effect of TCOEW on Annulus Time in Morris Water maze in Wister Rats
All values were expressed as mean ± SEM (n = 6). Data were analysed using one-way ANOVA followed by Dunnet's *post-hoc* test.
* = significance at $p < 0.05$ when compared with H_2O only. H_2O = Tap water, TCOEW = Treated Crude Oil Exploration Water

Effect of TCOEW on Brain Super Oxide Dismutase (SOD) Level in Wister Rats

The increased SOD shown by TCOEW treatment was statistically insignificant as shown in Figure 10 below.

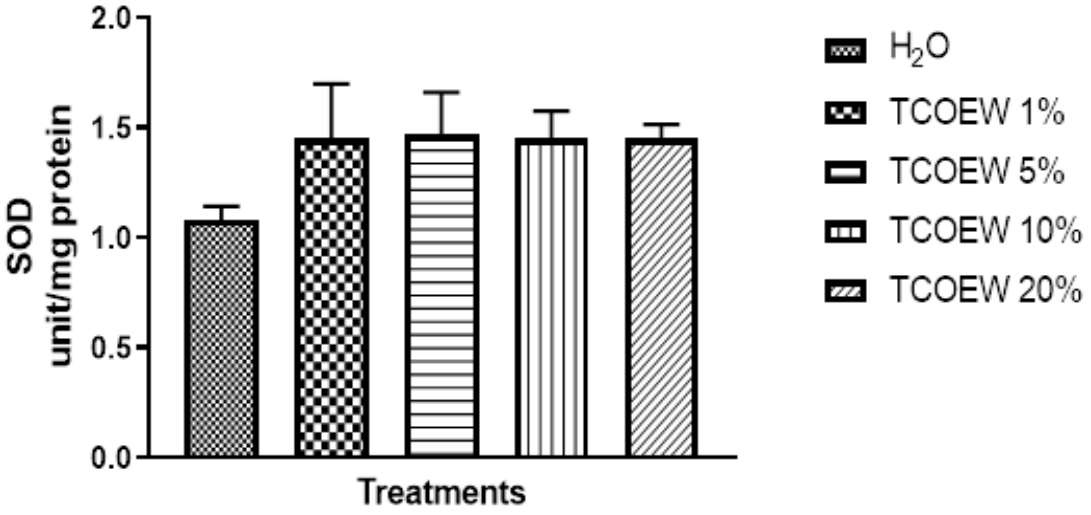


Fig 10: Effect of TCOEW on Brain Super Oxide Dismutase (SOD) Level in Wister Rats
All values were expressed as mean ± SEM (n = 6). Data were analysed using one-way ANOVA followed by Dunnet's *post-hoc* test.
* = significance at $p < 0.05$ when compared with H_2O only. H_2O = Tap water, TCOEW = Treated Crude Oil Exploration Water

Effect of TCOEW on Brain Catalase (CAT) Level in Wister Rats

All TCOEW treated group showed statistically insignificant increase in CAT level as shown in Figure 11 below.

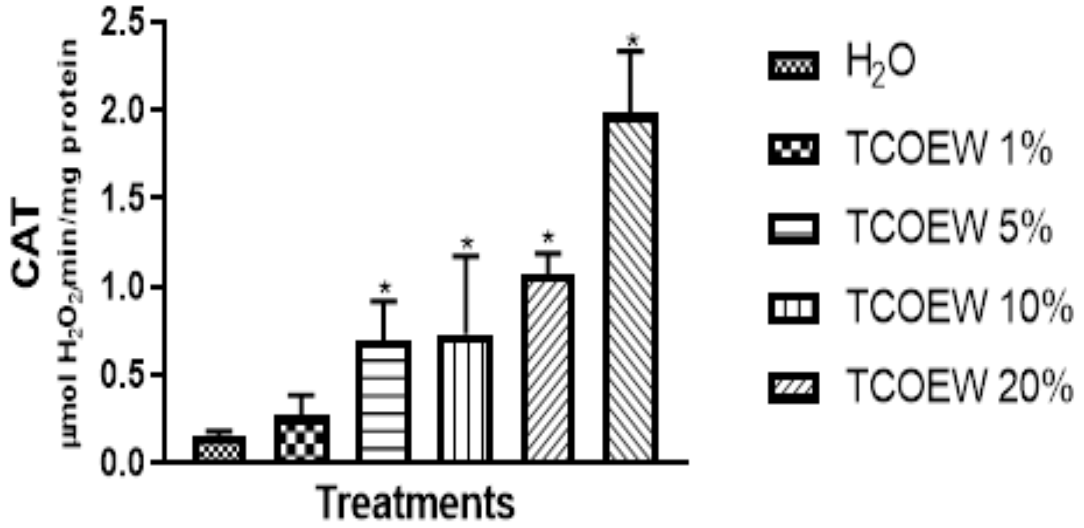


Fig 11: **Effect of TCOEW on Brain Catalase (CAT) Level in Wister Rats**All values were expressed as mean ± SEM (n = 6). Data were analysed using one-way ANOVA. H_2O = Tap water, TCOEW = Treated Crude Oil Exploration Water

Effect of TCOEW on Reduced Glutathione (GSH) in Wister Rats

The increased GSH shown by TCOEW was significant in TCOEW20% group; other groups showed increase GSH that was statistically insignificant as shown in Figure 12 below

.

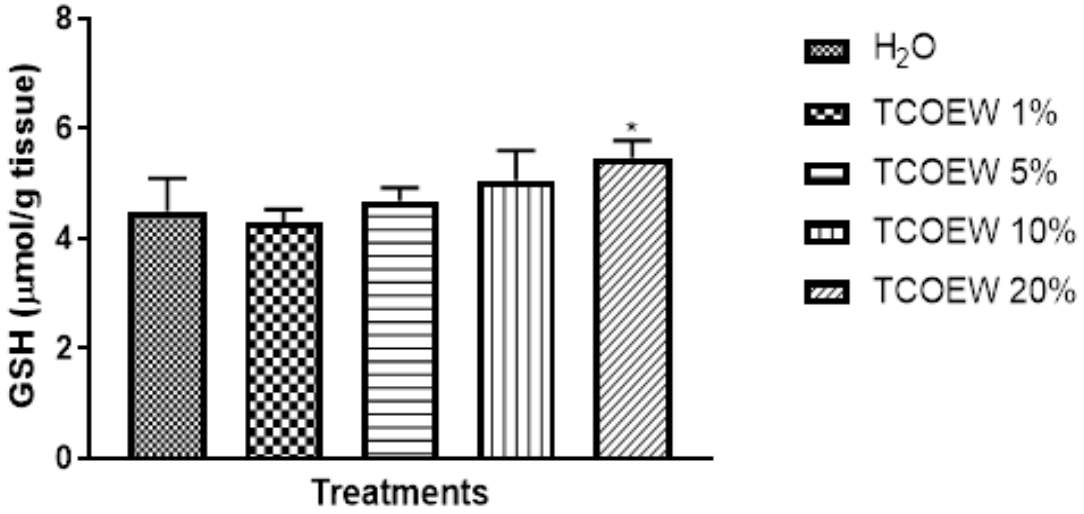


Fig 12: Effect of TCOEW on Brain Glutathione in Wister Rats

All values were expressed as mean ± SEM (n = 6). Data were analysed using one-way ANOVA followed by Dunnet's *post-hoc* test.

* = significance at $p < 0.05$ when compared with H_2O only. H_2O = Tap water, TCOEW = Treated Crude Oil Exploration Water

Effect of TCOEW on Brain Malondialdehyde (MDA) in Wister Rat

Exposure to TCOEW significantly increased in MDA level for all the groups except for TCOEW1% as shown in Figure 13 below.

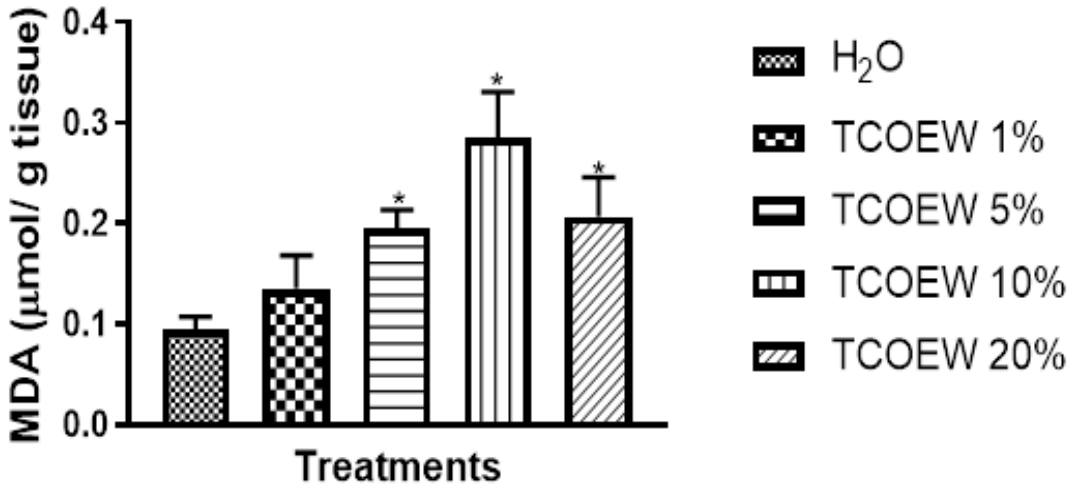


Fig 13: Effect of TCOEW on Brain Malonylaldehyde (MDA) in Wister Rats

All values were expressed as mean ± SEM (n = 6). Data were analysed using one-way ANOVA followed by Dunnet's *post-hoc* test.

* = significance at $p < 0.05$ when compared with H_2O. H_2O = Tap water, TCOEW = Treated Crude Oil Exploration Water

Effect of TCOEW on Nitrite level in Wister Rats

All TCOEW treated group showed statistically significant increase in nitrite level as shown in Figure 14 below.

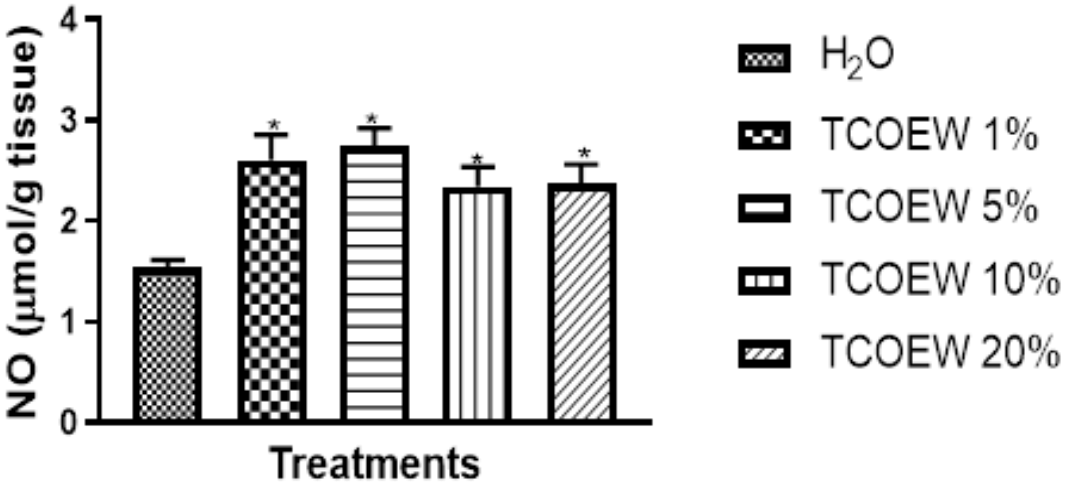


Fig 14: Effect of TCOEW on Nitrite level in Wister Rats

All values were expressed as mean ± SEM (n = 6). Data were analysed using one-way ANOVA followed by Dunnet's multiple comparison test

* = significance at $p < 0.05$ when compared with H_2O only. H_2O = Tap water, TCOEW = Treated Crude Oil Exploration Water

Effect of TCOEW on brain histology of treated Waster Rats

TCOEW 1%: There are numerous intact neurons (blue arrows). No visible lesion.

TCOEW 5%: There are multiple foci of neuronal necrosis (black arrows), with accumulation of glial cells (green arrows). The neutrophil appears normal. There are foci of cerebral haemorrhages (red arrows)

TCOEW10%: There are multiple foci of neuronal necrosis (black arrows), with focal accumulation of glial cells and neuronophagia (green arrows). The neutrophil appears normal.

TCOEW20%: There are numerous intact neurons (blue arrows). There are a few foci of neuronal necrosis (black arrow). There is moderate congestion of the cerebral blood vessels (red arrows)

H_2O (Control): There are numerous intact neurons (blue arrows).

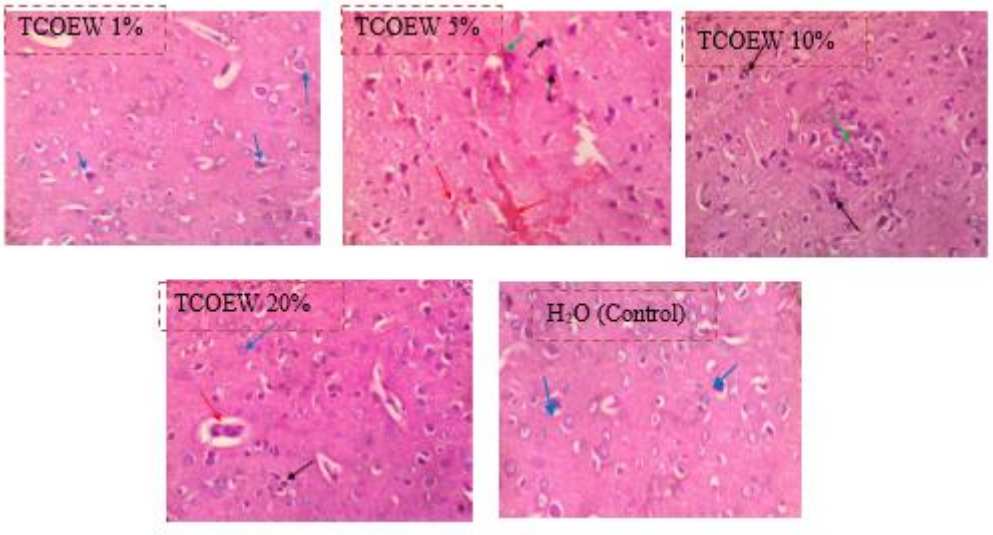


Fig 1: Effect of TCOEW on Brain histology of treated Male Wister Rats. Magnification: X100.
TCOEW: Treated Crude Oil Exploration Water

Discussion

In this study, behavioral paradigms (open field test, elevated plus maze, Morris water maze and novel object recognition test) after subjecting rats to 90 days exposure to produced water TCOEW), were explained by the modulation biochemical biomarkers and histology.

To the best of our knowledge, behavioral tests are not performed in most neurotoxicity studies, and these studies have not only failed to provide information on psychiatric aspect of the toxicant but they have not contributed much to knowledge on its central effects. Rearing and grooming in the open field box was used to determine the effect of produced water novelty induced behavior; elevated plus maze for anxiety; Morris water maze for memory, brain biochemical markers determined the oxidative status and histology revealed possible effect on neuronal architecture.

Exposure to produced water for ninety days reduced locomotion, rearing and grooming in the open field test. Rodent behavior in OFT is considered to mirror emotional reactivity and exploratory behavior (Gould *et al*., 2010). Grooming is primarily a normal behavior for care of the body by the animal, but it is increased in adapting to stress from fear or anxiety (Shaw *et al.,* 2007). Reduction in rearing and grooming brought about by TCOEW might be an indication of its inhibitory action. Locomotor activity is considered as a measure of alertness and its reduction indicates sedative activity (Lowry *et al.,* 2005). It has been reported that drugs that stimulate the CNS increase rearing and grooming behavior while drugs that depress the CNS inhibit the rearing and grooming; suggesting that TCOEW might have a CNS depression-like effect (Aderibigbe and Agboola, 2011). Nonetheless, exposure to mild stress such as a novel environment cause increased grooming behavior via activation of central D_1 receptor (Scalzitti *et al.,* 1999). Reduction in locomotion and novelty induced behaviors is suggestive of a possible CNS depressant activity (Murray *et al.,* 1996), such might be inferred as toxicity from chronic exposure to TCOEW.

The elevated plus maze naturally induce fear in the rodents due to balancing on a raised narrow platform and novel open space (Dawson and Tricklebank, 1995). It is a valid anxiety test, based on rodent aversion to height and open space, and their innate tendency to explore novel dark area (Akanmu *et al.,* 2011). Index of open arm avoidance (IOAA) was notably reduced by TCOEW exposed animals, although this does not imply anti-anxiety-like effect. Anxiety index difference not up to 10 points greater (anxiogenic) or less (anxiolytic) are not often acceptable (Akinpelu *et al.,* 2019). Higher units of IOAA infers observation of a reduction in percentage number of open arm entries and percentage time spent in open arm, and vice versa for lower unit IOAA. Rodents prefer closed arm but ventures into the open arm if they are less anxious under the influence of anxiolytic drugs, thus IOAA are lower than control.

Both anxiogenic and anxiolytic effects are mediated via GABAergic transmission which might not have been affected by TCOEW.

Novelty object recognition test (NORT) is an efficient and flexible test for learning and memory. It differs from other test of memory in that the time needed for the assay is quite short, and more than one brain regions and neurotransmitter systems are implicated (Moore et al., 2013). This makes it difficult to explain its neurobiology. Phosphodiesterase inhibitors enhance memory in NORT during acquisition and recall (Lueptow et al., 2015). Two cyclic nucleotides (cyclic adenosine monophosphate and cyclic guanosine monophosphate) have been reported to play a major role synaptic plasticity and memory formation. Time of novel object recognition as a parameter indicates memory performance in object recognition test, which is based on the increase in amount of time spent on exploration of novel objects in comparison with familiar objects. The time of novel object recognition is increased by inhibitors of PDEs due enhanced cGMP and cAMP intracellular signaling. In this test TCOEW treated groups showed reduced time spent exploring the novel object in NORT which depicts reduced cognition. This depict decreased recognition, and might be that chronic exposure to TCOEW decrease cognition.

The Morris water maze apparatus is used to test specific responsiveness to hippocampal lesions (D'Hooge and Deyn, 2001; Golchin *et al*, 2013) as hippocampal-dependent memory and spatial learning in rodents (Van Dam *et al*. 2006; Shabani *et al.*, 2012). The use of NORT has increased more than the other mazes because its results are robust, reliable and more widely replicated. Results obtained here are strongly correlated with hippocampus and NMDA receptor function. The escape time for all TCOEW treated groups shows marked reduction over the course of the learning trials. On probe day, annulus crossing and annulus time increased across the groups signifying the possibility of toxicity associated hippocampal region and NMDA receptor functions in memory.

Oxidative stress is the outcome of imbalance generation of free radicals and clearance by the antioxidant defense mechanism in cells. It has been involved in the pathology of several diseases of the brain because the relatively higher oxygen consumption due to cellular activity (Tabet *et al.,* 2000). The ability of many cells to resist oxidative stress is associated with high intracellular levels of anti-oxidants enzymes (Gupta and Sharma, 2006; Rodu and Ou, 2000). These enzymes metabolize the oxidative toxic intermediates and control the levels of lipid hydroperoxides to prevent cell damage by the free-radicals (Halliwell, 2015). Reduced glutathione (GSH) serves as an important endogenous anti-oxidant in the brain by scavenging harmful effect reactive species that are generated during different biochemical processes and chemical toxicant (Mahadik *et al.,* 1996). The superoxide dismutase (SOD) is an antioxidant enzyme that protects the cells against the damaging effects of reactive oxygen species, it specifically scavenges superoxide by catalysing its dismutation to H_20_2 and 0_2 (Hopkin, 2016). Catalase helps in fast decomposition of H_2O_2, it lowers the activation energy, the minimum barrier that H_2O_2 molecules have to decompose (Muller *et al.,* 1997). From this study, chronic exposure to TCOEW did not increase brain GSH, MDA, CAT and SOD statistically. It might thus be inferred that although it is not deleterious, exposure to TCOEW is not beneficial.

Conclusion

This study provides evidence that the treated crude oil exploration water might contain chemicals which on chronic exposure has effect on behavior, learning and memory, and anxiety. Although, further works needs to be done to ascertain the specific compounds responsible for this effect, the study justifies the importance for a call for strict compliance with the international standards for treatment of waste water before discharge into the water bodies.

References

1. Aderibigbe, A.O. and Agboola, O.I. 2011. Neuropharmacological profile of Struchium sparganophora (Linn) O. Ktze in mice. Asian Journal of Traditional Medicines, 6(3), 104-111.
2. Akanmu, M.A., Olowookere, T.A., Atunwa, S.A., Ibrahim, B.O., Lamidi, O.F., Adams, P.A., Ajimuda, B.O. and Adeyemo, L.E. 2011. Neuropharmacological effects of Nigerian honey in mice. African Journal of Traditional, Complementary and Alternative Medicines, 8(3).
3. Akinpelu, L.A., Adebayo, M.A., Fajana, A., Adeniyi-Ake, M.A., Ubogu, S.E. and Aminu, N.S. 2019. Phytochemical analyses, anxiolytic and anti-amnesic effect of methanol stem bark extract of Vitex doniana (Sweet) in mice. Nigerian Journal of Natural Products and Medicine, 23, 104-111.
4. Jimenez-Andrade, J.M., Bloom, A.P., Stake, J.I., Mantyh, W.G., Taylor, R.N., Freeman, K.T., Ghilardi, J.R., Kuskowski, M.A. and Mantyh, P.W. 2010. Pathological sprouting of adult nociceptors

in chronic prostate cancer-induced bone pain. Journal of Neuroscience, 30(44), 14649-14656.

5. Belovicova, K., Bogi, E., Csatlosova, K. and Dubovicky, M. 2017. Animal tests for anxiety-like and depression-like behavior in rats. Interdisciplinary toxicology, 10(1), 40.
6. Brown, L., Rosner, B., Willett, W.W. and Sacks, F.M. 1999. Cholesterol-lowering effects of dietary fiber: a meta-analysis. The American journal of clinical nutrition, 69(1), 30-42.
7. Carola, V., D'Olimpio, F., Brunamonti, E., Mangia, F. and Renzi, P. 2002. Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. Behavioural brain research, 134(1-2), 49-57.
8. Clinton, J.M. 2009. How has the science of early child development informed a child psychiatrist's practice? Paediatrics & Child Health, 14(10), 671-672
9. D'Hooge, R. and De Deyn, P.P. 2001. Applications of the Morris water maze in the study of learning and memory. Brain research reviews, 36(1), 60-90.
10. Dawson, G.R. and Tricklebank, M.D. 1995. Use of the elevated plus maze in the search for novel anxiolytic agents. Trends in pharmacological sciences, 16(2), 33-36.
11. Garey, J. and Paule, M.G. 2010. Effects of chronic oral acrylamide exposure on incremental repeated acquisition (learning) task performance in Fischer 344 rats. Neurotoxicology and teratology. 32(2), 220-225.
12. Gazali, A. K., Alkali, A. N., Mohammed, Y., Djauro, Y., Muhammed, D. D. and Kodomi M., 2017. Environmental Impact opf Produced Water and Drilling Waste Discharges from the Niger Delta Petroleum Industry. IOSR Journal of Engineering. 7:22-29.
13. Gould, C.M., Diella, F., Via, A., Puntervoll, P., Gemünd, C., Chabanis-Davidson, S., Michael, S., Sayadi, A., Bryne, J.C., Chica, C. and Seiler, M. 2010. ELM: the status of the 2010 eukaryotic linear motif resource. Nucleic acids research, 38(suppl_1), D167-D180.
14. Green, N., Alexander, H., Olson, A., Alexander, S., Shinnick, T.M., Sutcliffe, J.G. and Lerner, R.A. 1982. Immunogenic structure of the influenza virus hemagglutinin. Cell, 28(3), 477-487.
15. Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S. and Tannenbaum, S.R. 1982. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Analytical biochemistry, 126(1), 131-138.
16. Gupta, V.K. and Sharma, S.K., 2006. Plants as natural antioxidants.
17. Haber, E., Danenberg, H.D., Koroukhov, N., Ron-El, R., Golomb, G. and Schachter, M. 2009. Peritoneal macrophage depletion by liposomal bisphosphonate attenuates endometriosis in the rat model. Human Reproduction, 24(2), 398-407.
18. Hall, C.S., 1934. Drive and emotionality: factors associated with adjustment in the rat. Journal of Comparative Psychology, 17(1), p.89.
19. Halliwell, L.M, 2015. Protein engineering utilising single amino acid deletions within Photinus pyralis firefly luciferase (Doctoral dissertation, Cardiff University).
20. Hopkin, K. 2016. Inner workings: A big role for a microbial parasite. Proceedings of the National Academy of Sciences, 113(16), 4236-4237.
21. Jollow, D., Thorgeirsson, S.S., Potter, W.Z., Hashimoto, M. and Mitchell, J.R. 1974. Acetaminophen-induced hepatic necrosis. Pharmacology, 12(4-5), 251-271.
22. Jollow, D.J., Mitchell, J.R., Zampaglione, N. and Gillette, J.R. 1974. Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3, 4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology, 11(3), 151-169.
23. LoPachin, R.M. 2004. The changing view of acrylamide neurotoxicity. Neurotoxicology, 25(4), 617-630.
24. LoPachin, R.M. and Gavin, T. 2012. Molecular mechanism of acrylamide neurotoxicity: lessons learned from organic chemistry. Environmental health perspectives, 120(12), 1650-1657.
25. Lowry, C.A., Johnson, P.L., Hay-Schmidt, A., Mikkelsen, J. and Shekhar, A. 2005. Modulation of anxiety circuits by serotonergic systems. Stress, 8(4), 233-246.
26. Mahadik, S.P. and Mukherjee, S. 1996. Free radical pathology and antioxidant defense in schizophrenia: a review. Schizophrenia research, 19(1), 1-17.
27. Misra, H.P. and Fridovich, I. 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological chemistry, 247(10), 3170-3175.
28. Moore, K.J., Sheedy, F.J. and Fisher, E.A. 2013.

Macrophages in atherosclerosis: a dynamic balance. Nature Reviews Immunology, 13(10), 709-721.

29. Müller, M., Strand, S., Hug, H., Heinemann, E.M., Walczak, H., Hofmann, W.J., Stremmel, W., Krammer, P.H. and Galle, P.R. 1997. Drug-induced apoptosis in hepatoma cells is mediated by the CD95 (APO-1/Fas) receptor/ligand system and involves activation of wild-type p53. The Journal of clinical investigation, 99(3), 403-413.
30. Murray, L., Stanley, C., Hooper, R., King, F. and Fiori-Cowley, A. 1996. The role of infant factors in postnatal depression and mother-infant interactions. Developmental Medicine & Child Neurology, 38(2), 109-119.
31. Reppa, I., Williams, K.E., Greville, W.J. and Saunders, J. 2020. The relative contribution of shape and colour to object memory. Memory & Cognition, 48(8), 1504-1521.
32. Rodu, B. and Ou, B, 2000. The antioxidant properties of tobacco. Tobacco Science, (44 (44)), 71-73.
33. Scalzitti, J.M., Cervera, L.S., Smith, C. and Hensler, J.G. 1999. Serotonin2A receptor modulation of D1 dopamine receptor-mediated grooming behavior. Pharmacology Biochemistry and Behavior, 63(2), 279-284.
34. Shabani, M., Nazeri, M., Parsania, S., Razavinasab, M., Zangiabadi, N., Esmaeilpour, K. and Abareghi, F. 2012. Walnut consumption protects rats against cisplatin-induced neurotoxicity. Neurotoxicology, 33(5), 1314-1321.
35. Shaw, P., Eckstrand, K., Sharp, W., Blumenthal, J., Lerch, J.P., Greenstein, D.E.E.A., Clasen, L., Evans, A., Giedd, J. and Rapoport, J.L. 2007. Attention-deficit/hyperactivity disorder is characterized by a delay in cortical maturation. Proceedings of the national academy of sciences, 104(49), 19649-19654.
36. Sinha, R.N. 1971. Fungus as food for some stored-product insects. Journal of Economic Entomology, 64(1), 3-6.
37. Tabet, N., Mantle, D. and Orrell, M. 2000. Free radicals as mediators of toxicity in Alzheimer's disease: a review and hypothesis. Adverse drug reactions and toxicological reviews, 19(2), 127-152.
38. Tellez, M.R., Khan, I.A., Kobaisy, M., Schrader, K.K., Dayan, F.E. and Osbrink, W. 2002. Composition of the essential oil of Lepidium meyenii (Walp.). Phytochemistry, 61(2), 149-155.
39. Van Dam, R.M., Willett, W.C., Manson, J.E. and Hu, F.B. 2006. Coffee, caffeine, and risk of type 2 diabetes: a prospective cohort study in younger and middle-aged US women. Diabetes care, 29(2), 398-403.
40. Vorhees, C.V. and Williams, M.T. 2014. Value of water mazes for assessing spatial and egocentric learning and memory in rodent basic research and regulatory studies. Neurotoxicology and Teratology, 45, 75-90.