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# Neuromuscular Toxicity Profiling of Synthetic-Based Drilling Fluids in Bushman's Rabbits from The Niger Delta, Nigeria

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***ABSTRACT***
*Ester Based Mud, a Synthetic-Based drilling fluid used in oil and gas exploration within the Niger Delta pose potential risks to terrestrial food chains through bioaccumulation. This study evaluated the sublethal neuromuscular toxicity of synthetic-based drilling fluids in Bushman's rabbits (Bunolagus monticularis) using biochemical indices. A randomized definitive experiment was conducted with twenty-four rabbits acclimated for fourteen days and subsequently exposed to 0 (control), 300, 500, 700, and 900 mg/L of drilling fluid for twenty-eight days. Brain and muscle tissues were harvested and analyzed for electrolyte ($Na^+$, $K^+$, $Cl^-$, and $Mg^{2+}$) and metabolite (total protein, albumin, urea, and creatinine) concentrations. Results showed significant alterations ($p < 0.05$) in sodium and chloride ions in both brain and muscle tissues, accompanied by fluctuating potassium levels, while magnesium remained largely unaffected. Metabolite analysis revealed no significant changes in total protein, albumin, and urea; however, creatinine levels declined significantly in muscle tissue. These findings demonstrate that synthetic-based drilling fluids induce neuromuscular biochemical disturbances, with potential implications for motor coordination, osmoregulation, and renal function. The study highlights the need for stricter regulation and continuous monitoring of drilling fluid use to safeguard wildlife and human food chains in the Niger Delta region of Nigeria.*

***Key words:*** *Bunolagus monticularis, Synthetic Drilling Fluid, Electrolyte, Metabolites, Neuromuscular.*

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## I. Introduction

Oil exploration activities increase the likelihood of environmental contamination by drilling fluids. Chronic exposure in wildlife such as Bushman's rabbit may result in reduced mobility, impaired predator avoidance, poor foraging efficiency, and decreased reproductive fitness. Neuromuscular dysfunction can significantly affect ecosystem balance (Patani, et al., 2020).

Food security has emerged as a multifaceted global challenge that intersects with nearly all areas of agricultural practice, including crop cultivation, livestock production, and aquaculture or pisciculture (Vincent-Akpu & Sikoki, 2013). In the Niger Delta region of Nigeria, livestock rearing and hunting constitute important sources of animal protein and household income. However, these livelihoods are increasingly under threat due to the bioaccumulation and environmental contamination associated with drilling fluids and their wastes arising from intensified and poorly regulated oil exploration activities (Patani & Inyang, 2024). Consequently, pollutants introduced into the environment have infiltrated the human food chain, contributing to biodiversity loss and persistent environmental degradation across the region.

Drilling fluids play a central role in oil and gas exploration, as they are circulated within the wellbore to transport drill cuttings to the surface, enhance fluid rheological properties, control filtration loss, and facilitate efficient penetration through diverse subsurface formations (Talalay et al., 2014). In Nigeria's Niger Delta, oil and gas exploration and production activities occur extensively, given the sector's critical contribution to the national economy. Despite these economic benefits, such operations have generated adverse ecological consequences, including contamination of terrestrial and aquatic food chains, leading to environmental stress and ecosystem imbalance (Talalay et al., 2014).

The success of drilling operations largely depends on accurate geological information, favorable reservoir conditions, and appropriate drilling strategies. Accordingly, the primary objectives of drilling operations include reaching the intended target formation safely, within the shortest feasible timeframe and at minimal operational cost, while effectively addressing sampling, evaluation, and technical constraints (Chemwotei, 2011). A key functional requirement of drilling fluids is their ability to lubricate the drill bit and efficiently transport rock cuttings from the wellbore as drilling progresses.

Traditionally, drilling fluids are classified based on their base materials, such as air-based, water-based, or oil-based systems. However, contemporary drilling operations globally favor synthetic-based and oil-based fluids due to their perceived lower toxicity and superior performance characteristics (Chemwotei, 2011). Notwithstanding these advantages, evidence suggests that these drilling muds and formulations may exert harmful effects on ecosystems and contribute to contamination of food chains when inadequately managed.

This study therefore is aimed at evaluating the neuromuscular toxicity of synthetic-based drilling fluid (Ester-Based Mud) in Bushman's Rabbits by assessing its effects on metabolites, such as total protein, albumin, urea and creatinine, as well as electrolytes, such as sodium, potassium, chloride, and magnesium ions in the brain and muscle.

## II. Literature Review

The Niger Delta ecosystem of Nigeria, due to its unique terrain is blessed with varying species of forest resource, including animals (Patani et al., 2020). It is a fertile ground for hunting and animal husbandry, which is one of the main economic stay of the people of the area. In recent past, oil drilling and exploration activities in the area have caused debilitating impact on the region due to the toxic wastes generated from the use of drilling fluids, thereby destroying the ecosystem, hunting and animal husbandry in the area. These operations by the Oil and Gas companies have exacerbated the destructive influence of human activities on the aquatic ecosystem within the area, thus resulting in chronic stress conditions with negative impact on aquatic lives (Oil, Gas & Petrochemical, 2003).

In Oil and Gas operations, drilling fluids, also referred to as drilling muds, are used to lubricate and cool the drilling apparatus, transport drill cuttings to the surface and seal off porous geologic formations (Sahar & Abouzar, 2009). Drilling fluids typically consist of Bentonite and a range of additives mixed with fresh water or hydrocarbons. But the irony is that after most drilling operations, these drilling muds and their wastes are left to the environment without adequate treatment and they eventually find their way into aquatic environment, causing severe pollution impact on aquatic lives, including fish. In most cases, drilling fluids and their wastes are left in temporary holding-pits during onshore drilling operations and sometimes dumped directly into surrounding waters such as swamps, rivers and oceans during off-shore drilling (Sahar & Abouzar, 2009). But these effects or impacts can be mitigated or controlled by carrying out effective bioassay testing to evaluate and study the toxicity of drilling fluids and their wastes to aquatic lives (biota), which is the core of this research.

Drilling fluids are fluids used to aid the drilling of boreholes into the earth. Drilling fluids performs very important functions in Oil and Gas drilling and horizontal directional drilling (OGP, 2003). It is also a specially compounded liquid that circulates through the wellbore during rotary drilling operations. Drilling fluid is a complex system that contains a fluid phase, solid phase and a chemical phase (Sahar & Abouzar, 2009). Other than the fluid and the solid phases, different types of chemicals and polymers are been used in designing a drilling mud in the chemical phase to develop the key properties of the mud that meet some functional requirements such as mud rheology, density, mud activity and fluid loss control etc. Therefore, the current varieties of fluid additives thus reflect the complexity of mud systems in use today, which is daily increasing as more difficult and challenging drilling conditions are encountered.

Synthetic Based Drilling Fluids (SBDFs) are by far the most commonly used fluids for both onshore and offshore drilling operations (API, 2003). It can be provided with various additives, such as fluid loss control agents, corrosion inhibitors, weighting materials, and viscosifiers, to develop the key properties of the mud to meet some functional requirements (API, 2003). Even though the overall environmental impact of mud additives in SBMs are minimal, it can contaminate and harm the marine life that inhabits the surrounding waters where drilling operations take place (Jerry & Neff, 2005).

Over the years, due to delayed realization of the environmental impact of mud additives such as chemicals, polymers, salt water and oil-based fluids, little or no studies have been carried out to assess the environmental impact of these fluids on the ecosystem at the early stage of drilling. However, recent studies have shown that some of the synthetic based mud (SBM) additives that were acceptable from an environmental point

of view decades ago are now no longer acceptable for current and future drilling operations in environmentally sensitive areas (Sahar & Abouzar, 2009).

This is one of the reasons why there is the need for frequent studies and assessment of Synthetic Based Drilling Muds (SBM) and their additives in other to properly select the composition that would be safe as well as standards that defines the disposal guidelines of these muds all over the world (Amanullah, 2007). The recent clamour by governments all over the world to improve on environmental laws to curb the menace of air pollution, clean water, hazardous waste disposal, as well as the control of occupational health and safety hazards have charted a new course for the petroleum industry to begin to re-evaluate all aspects of drilling and production. These changes have greatly affected drilling fluid additive choices.

From available literatures, drilling fluids and their wastes have shown to have caused a lot of negative effects to aquatic ecosystem such as fish mortality and smothering. The later in most cases leads to oxygen depletion as well as increased particles suspension in water mainly due to the release of Water Based Drilling Muds and their additives during oil drilling operations (Saasen, et al., 2001). Other debilitating effects include potential damage to aquatic invertebrate population and alteration in feeding and spawning grounds (Soegianto et al., 2008).

Synthetic Based Drilling Fluids, their additives and wastes have also been reported in literature to have some negative impacts on man, some of which include skin irritation, contact dermatitis, coughing as well as nausea etc. But these effects are mainly dependent on the type, dosage and exposure duration of the chemical (Ezemonye et al., 2008). Drilling muds and additives get easily released into aquatic ecosystems in the Niger Delta area through accidental discharge, poor waste management and handling, as well as poor management of drill cuttings during the drilling process (Ezemonye et al., 2008). These fluids, additives and wastes are chemically toxic or physically harmful to marine flora and fauna.

Various studies have also shown that the growth of flora and fauna have been affected by toxic heavy metal contaminations in the environment (Silva et al., 2009), most of which are components of drilling mud additives. For instance, Mojtahid and associates, (2006) demonstrated that oily drill cutting discharges affected 750 $m^2$ of area from a disposal site in Congo and West Africa, while Morvan (2004) demonstrated that the formation of intertidal mudflats was impacted due to oil spillages. These discharges could have both short and long-term adverse impacts on aquatic organisms and wildlife present near the shoreline (McCay et al., 2004).

Sublethal ecotoxicity testing is generally used to predict the toxicity of Xenobiotics, like Synthetic Based Drilling Fluid, in the environment. In most cases, toxicity testing with fish species is aimed at assessing the possible risk to similar species or other live forms in various ecosystems including aquatic ecosystem, determination of possible water quality criteria for regulatory purposes and to establish correlation with acute testing of other species.

Food security is also another complex issue, cutting across virtually all aspects of agricultural practice, including crop production, livestock breeding and fish farming or aquaculture (Vincent-Akpu & Sikoki, 2013). Thus, animal products are generally regarded as high risk commodities due to possible bioaccumulation and contamination by Xenobiotics like drilling fluids and its wastes, natural toxins and even pathogens (Yousuf et al., 2008). Thus, using animal models in scientific or eco-toxicological studies have been given a positive backing for the purpose of validating existing literature and procedures (Miranda et al., 2005). This is aimed at creating innovations that are backed by scientific principles to help reduce environmental contamination by Xenobiotics, resulting in food poisoning (Calasans-Maia et al., 2008).

Therefore, findings from this study will help provide a basis for assessing the environmental acceptability of synthetic based drilling fluids and its use by the Oil and Gas industry in Nigeria, as well as guide prudent decision making to minimize damage on the environment as well as prevent food poisoning.

## III. Materials and Methods

### 3.1 Experimental Design

A completely randomized experimental design was used for the experiment. A total of twenty-four (24) Bushman's rabbits (Bunolagus monticularis) were used in this study. The animals were randomly assigned to treatment groups and exposed to sublethal concentrations of synthetic-based drilling fluid for a period of 21 days using a completely randomized definitive experimental design.

### 3.2 Source of Rabbits and Rabbitory

Bushman's rabbits (*Bunolagus monticularis*) were procured from Kester livestock Farms at Ogboloma Town, Yenagoa, Bayelsa State, Nigeria. Following procurement, the animals were transported in ventilated plastic cages to the animal house (rabbitory) of the Department of Biology, Faculty of Science, Bayelsa Medical University, Bayelsa State, Nigeria, where the experiment was conducted. The rabbitory have a total of twelve (12) hutches, with each having a dimension of 90cm*75cm*120cm with doors. Leather coverage was made available to ensure rabbits are shielded from excessive cold during bad weather conditions and in the night hours to avoid unexpected death. The rabbitory was made of wooden members and aluminum roofing sheets. An equal dark and light conditions where maintained at all times during the experimental period.

### 3.3 Handling of Rabbits

Rabbits were handled with care and gloves were always put on when touching rabbits. They were always lifted or carried by the skin in the dorso-cervical area, a fold of muscles at the dorsum of the upper part of the neck. Picking rabbits up by the ears was deliberately avoided as there is much chance of leading to cervical luxation and demise.

### 3.4 Collection of Synthetic Drilling Fluid (Synthetic Ester Mud) and Preparation

Synthetic Esther Mud is formulated from synthetic esters, derived from fatty acids and alcohols that are readily biodegradable and have low toxicity. It is a common type of synthetic-based drilling fluid. Synthetic Ester Mud was obtained directly from an active drilling rig site located within Yenagoa metropolis, Bayelsa State, Nigeria. At the time of collection, the drilling fluid had already been formulated and prepared in line with operational requirements that meets company and industry standard. The drilling fluid was collected into plastic bottles well labelled and cocked and was transported to the laboratory for the experiment. Before using the drilling fluid, the chemical was thoroughly agitated and homogenized before the required millilitres were measured out for the experiment.

### 3.5 Acclimatization

Prior to the commencement of the definitive exposure experiment, all experimental rabbits were acclimatized in the animal house for a period of fourteen (14) days. The acclimatization process was undertaken to allow the animals to adjust to laboratory conditions, minimize handling as well as environmental stress, physiological stabilization and detoxification before toxicant exposure.

### 3.6 Range-Finding Test

After the acclimatization period, a preliminary range-finding test was conducted to determine appropriate sublethal exposure concentrations for the main experiment. Four (4) Rabbits were randomly selected and exposed to four different nominal concentrations of the drilling fluid (0.30, 0.50, 0.70, and 0.90 mL). Each concentration was prepared by dissolving the specified volume of drilling fluid in 2.0 L of tap water. The range-finding test was carried out over a period of seven (7) days, following the procedure described by Inyang (2008). The outcome of this preliminary assessment served as a guide for selecting sublethal concentrations suitable for the definitive exposure study.

The exposure concentrations obtained during the range-finding test, initially expressed in millilitres (mL), were subsequently converted and expressed as milligrams per litre (mg/L) for use in the main experiment, using the appropriate conversion formula.

$$C = \frac{C_s\,(ml) \times (mg)}{V_w\,(L)} \quad \text{.....} \quad \text{E.q -2.1.}$$

Where:

C: (Concentration of drilling fluid used in the experiment).

$C_s$: (Arbitral amount of solute - drilling fluid used for the trial test in millilitre).

mg: (Conversion of millilitre (ml) to milligram (mg). This is achieved by multiplying the millilitre (ml) by 1000). That is; 1ml = 1000 mg.

$V_w$: (Volume of water used to make up the test concentration – 2.0 L).

Therefore, the four (4) sublethal concentrations used in the definitive or main experiment were viz; 300 mg/L, 500 mg/L, 700 mg/L and 900 mg/L.

### 3.7 Definitive (Main Sublethal Exposure) Test

The experiment was completely randomized during the definitive sublethal exposure period. The study comprised five experimental groups, consisting of one unexposed control group and four treatment groups with four replicates in each group. Each treatment group was exposed to a specific concentration of synthetic-based drilling

fluid: 300, 500, 700, and 900 mg/L, while the control group received no toxicant exposure, and all groups were in quadruplicate as stated earlier.

Out of the twenty-four Bushman's rabbits for the experiment, twenty (20) were randomly selected for the definitive test, of which sixteen (16) were randomly assigned to the treatment groups and four (4) served as controls. The exposure period lasted for twenty-eight (28) consecutive days.

### 3.8 Administration of Treatment and Feeding

Throughout the experiment, the sublethal treatment concentrations were constantly measured and mixed with 2.0 L of pipe born water in a metal plate and orally administered through the drinking water. This was renewed at 11:00 hours daily to ensure constant exposure concentrations in line with renewal bioassay experiments. Rabbits were fed with standard poultry feeds (pelletized), and equal photoperiod conditions (12 h light/12 h dark) were maintained at ambient laboratory temperature. Feeding was withdrawn 24 hours prior to the termination of the experiment to minimize post-prandial interference with biochemical assessments.

### 3.9 Termination of Experiment

At the end of the 28 days exposure period, experimental animals were transported in plastic cages, well labelled to the laboratory for termination. In the laboratory, each rabbit from each group was brought out from the cage by holding the skin in the dorso-cervical area, a fold of muscles at the dorsum of the upper part of the neck. Handling rabbits by the ears was deliberately avoided in order to prevent cervical luxation and demise.

Each experimental rabbit for dissection was carefully placed on a sterile dissecting board in a work bench, and humanely sacrificed by cervical trauma using a ceramic pestle to immobilize them. During sample handling and processing, standard laboratory safety protocols were strictly observed, including the use of personal protective equipment (laboratory coats, gloves, and face masks) and routine sterilization procedures to prevent contamination and ensure data integrity.

### 3.10 Sample Collection and Preparation

Immediately thereafter, samples of brain and skeletal muscle tissues were excised using sterile dissecting knife. The harvested tissues were weighed in an electronic weighing balance, crushed in a ceramic mortar using a pestle, and homogenized in a mixture of 0.5 mL perchloric acid and deionized water. The homogenates were decanted into labelled centrifuge tubes and centrifuged at 3000 revolutions per minute (rpm) for 15 minutes to obtain clear supernatants. The various supernatants were then decanted into well labelled sample bottles and stored in a refrigerator with regulated temperature between $2^{o}$-$8^{o}$C, before sending to the chemical laboratory, Federal Medical Centre, Yenagoa, Bayelsa State for analysis.

### 3.11 Procedure for Biochemical Analysis in the Chemical Laboratory

In the chemical laboratory of Federal Medical Centre (FMC), Yenagoa, Bayelsa State, Nigeria, biochemical analysis of electrolytes and metabolites followed standard laboratory analysis procedures.

### 3.12 Procedure for Metabolites Analysis

Biochemical analyses of metabolites were conducted using an automated clinical chemistry analyzer (Mindray BS-240, Shenzhen, China). The analyzer was operated in accordance with the manufacturer's instructions. Absorbance measurements were obtained within the visible spectrum at metabolite-specific wavelengths. Routine laboratory equipment such as centrifuges, adjustable micropipettes, incubators maintained at 37 °C, and a UV–visible spectrophotometer were employed as required.

Commercially prepared diagnostic reagent kits were used for all metabolite estimations. Urea was assessed by using the urease method, creatinine by enzymatic and alkaline picrate methods, total protein by the biuret reaction, albumin by bromocresol green dye-binding. All reagents were of analytical grade and supplied by reputable diagnostic manufacturers. The analyzer was calibrated using manufacturer-provided multi-analyte calibrators prior to sample analysis. Internal quality control sera at normal and pathological concentration levels were analyzed alongside experimental samples to monitor accuracy and precision. Analytical runs were considered valid only when quality control results were within acceptable ranges. Samples and reagents were equilibrated to room temperature prior to analysis. Predefined volumes of samples were aspirated by the analyzer and reacted with corresponding reagent mixtures. The reactions were incubated at 37 °C for the specified time, and absorbance readings were automatically recorded. Concentrations of metabolites were calculated by the analyzer software using established calibration factors. All generated data were reviewed for analytical consistency and instrument flags, and only validated results were included in the final dataset for statistical analysis.

### 3.13 Procedure for Electrolytes Analysis

Concentrations of sodium ($Na^+$), potassium ($K^+$), chloride ($Cl^-$), and magnesium ($Mg^{2+}$) were determined using the ion-selective electrode (ISE) technique. This analytical method is based on selective permeability of electrode membranes to specific ions, generating electrical potentials proportional to ion activities, which are translated into concentration values by the analyzer. Electrolyte measurements were conducted using an automated electrolyte analyzer based on direct ISE principles (Mindray BS-300 Electrolyte Analyzer, Shenzhen, China). The system automatically aspirates samples and reports electrolyte concentrations in mmol/L. Additional laboratory equipment included a bench centrifuge, adjustable micropipettes (10–1,000 µL), sample cups, and a laboratory refrigerator.

The analyzer was fitted with sodium-, potassium-, and chloride-selective electrodes and an internal reference electrode. Manufacturer-supplied electrolyte calibrators at low and high concentration levels were used for instrument calibration. Commercially prepared quality control sera at normal and pathological concentration levels were analyzed to ensure analytical reliability. All reagents were used according to the manufacturer's instructions. Instrument calibration was carried out prior to sample analysis and subsequently as recommended by the manufacturer. Analytical runs were accepted only when quality control values were within acceptable limits.

Samples and reagents were equilibrated to room temperature prior to analysis. Defined volumes of serum were aspirated automatically by the analyzer, and electrolyte concentrations were calculated based on measured electrode potentials. Results were displayed electronically and recorded. Results were examined for analytical consistency and instrument flags. Samples with questionable results were reanalyzed, and only validated data were used for statistical analysis.

### 3.14 Statistical Analysis

All data obtained from the experiment were analyzed using the Statistical Package for Social Sciences (SPSS), version 20.8. One-way analysis of variance (ANOVA) was performed to evaluate differences among experimental groups. Results were expressed as mean ± standard deviation. Where statistically significant differences were detected, Turkey's Honestly Significant Difference (HSD) post hoc test was applied to compare group means and determine the level of statistical separation among treatments.

## IV. Results And Discussions

### 4.1 Results

The observed biochemical disruptions as observed in Tables 1-4 of this study suggest that synthetic-based drilling fluids, though often considered environmentally safer than oil-based alternatives, can exert significant neuromuscular toxicity under sublethal exposure. Chronic exposure in natural settings could therefore result in impaired locomotion, feeding efficiency, and survival of terrestrial herbivores, with cascading effects on human food security.

The results of the analysis of electrolyte ($Na^+$, $K^+$, $Cl^-$, and $Mg^{2+}$) and metabolite (total protein, albumin, urea, and creatinine) in the brain and muscles of exposed rabbits as presented in Tables 1-4 of this study revealed significant alterations ($p < 0.05$) in sodium and chloride ions in both brain and muscle tissues, accompanied by fluctuating potassium levels, while magnesium remained largely unaffected. Metabolite analysis revealed no significant changes in total protein, albumin, and urea; however, creatinine levels declined significantly in muscle tissue.

**Table 1.** Mean levels of Electrolytes in the Brain of *Bunolagus monticularis* exposed to Synthetic Based Drilling Fluid for 21 days (mean ±SD).

| Conc. of Synthetic Based Drilling Fluid (mg/L) | $Na^+$ (mmol/L) | $K^+$ (mmol/L) | $Cl^-$ (mmol/L) | $Mg^{2+}$ (mmol/L) |
|---|---|---|---|---|
| 0.00 | $13.15\pm3.0^{c}$ | $8.25\pm2.1^{c}$ | $10.25\pm5.0^{c}$ | $0.60\pm0.2^{a}$ |
| 300 | $5.55\pm31.3^{a}$ | $12.03\pm3.4^{a}$ | $21.23\pm2.4^{a}$ | $0.33\pm0.4^{a}$ |
| 500 | $7.48\pm1.3^{d}$ | $11.23\pm2.0^{b}$ | $8.43\pm2.4^{c}$ | $0.23\pm0.1^{a}$ |
| 700 | $3.15\pm1.2b^{cd}$ | $6.35\pm1.0^{cb}$ | $5.13\pm1.3^{d}$ | $0.24\pm0.3^{a}$ |
| 900 | $18.53\pm5.0^{b}$ | $2.10\pm3.1^{d}$ | $14.18\pm1.7^{b}$ | $0.11\pm0.2^{a}$ |

Different superscript indicate a significant variation ($p<0.05$) $a^*$, b*, c*, d*.

**Table 2.** Mean levels of electrolytes in the Muscle of *Bunolagus monticularis* exposed to Synthetic Based Drilling Fluid for 21 days (mean ±SD).

| Conc. of Synthetic Based Drilling Fluid (mg/L) | $Na^+$ (mmol/L) | $K^+$ (mmol/L) | $Cl^-$ (mmol/L) | $Mg^{2+}$ (mmol/L) |
|---|---|---|---|---|
| 0.00 | $3.28\pm2.1^{d}$ | $2.10\pm3.1^{b}$ | $11.23\pm2.5^{a}$ | $0.21\pm0.3^{a}$ |

| | | | | |
|---|---|---|---|---|
| 300 | 3.17±3.2$^{e}$ | 3.50±1.3$^{a}$ | 9.13±1.2$^{a}$ | 0.23±0.3$^{a}$ |
| 500 | 6.35±4.1$^{c}$ | 2.09±2.8$^{d}$ | 9.18±4.2$^{b}$ | 0.19±0.3$^{a}$ |
| 700 | 11.13±3.2$^{b}$ | 2.11±2.2$^{c}$ | 6.7±1.2$^{bc}$ | 0.10±0.2$^{a}$ |
| 900 | 14.15±2.1$^{a}$ | 2.11±1.3$^{b}$ | 2.12±1.4$^{c}$ | 0.12±0.2$^{a}$ |

Different superscript indicated a significant variation ($p<0.05$) a$^{*}$, b*, c*, d*.

**Table 3.** Mean levels of metabolites in the Brain of *Bunolagus monticularis* exposed to Synthetic Based Drilling Fluid for 21 days (mean ±SD).

| Conc. of Synthetic Based Drilling Fluid (mg/L) | T.P (g/L) | ALB (g/L) | UREA (mmol/L) | CREAT (µmol/L) |
|---|---|---|---|---|
| 0.00 | 2.10±0.2$^{a}$ | 3.10±0.2$^{a}$ | 0.10±0.1$^{a}$ | 14.10±01.2$^{b}$ |
| 300 | 3.11±1.2$^{a}$ | 3.05±0.2$^{a}$ | 0.11±0.2$^{a}$ | 12.05±01.1$^{a}$ |
| 500 | 2.12±0.3$^{a}$ | 3.05±0.1$^{a}$ | 0.10±0.3$^{a}$ | 13.10±02.2$^{d}$ |
| 700 | 2.13±0.3$^{a}$ | 2.02±0.1$^{a}$ | 0.03±0.3$^{a}$ | 12.01±04.3$^{c}$ |
| 900 | 2.11±1.3$^{a}$ | 3.10±0.3$^{a}$ | 0.03±0.2$^{a}$ | 14.02±05.2$^{b}$ |

Different superscript indicated a significant variation ($p<0.05$) a$^{*}$, b*, c*, d*.

**Table 4.** Mean levels of metabolites in the Muscle of *Bunolagus monticularis* exposed to Synthetic Based Drilling Fluid for 21 days (mean ±SD).

| Conc. of Synthetic Based Drilling fluid (mg/L) | T.P (g/L) | ALB (g/L) | UREA (mmol/L) | CREAT (µmol/L) |
|---|---|---|---|---|
| 0.00 | 2.10±1.1$^{a}$ | 0.05±1.1$^{a}$ | 0.10±0.1$^{a}$ | 12.15±1.2$^{a}$ |
| 300 | 2.4±0.2$^{a}$ | 0.05±0.2$^{a}$ | 0.15±0.3$^{a}$ | 13.12±1.0$^{d}$ |
| 500 | 2.20±0.1$^{a}$ | 0.01±1.2$^{a}$ | 0.10±0.3$^{a}$ | 15.10±1.3$^{b}$ |
| 700 | 2.10±0.3$^{a}$ | 0.05±0.2$^{a}$ | 0.03±0.2$^{a}$ | 17.12±0.3$^{bc}$ |
| 900 | 2.2±0.2$^{a}$ | 0.02±0.1$^{a}$ | 0.03±0.3$^{a}$ | 28.11±3.0$^{c}$ |

Different superscript indicated a significant variation ($p<0.05$) a$^{*}$, b*, c*, d*.

### 4.2. Discussions

The findings in Table 1-4 provides compelling biochemical evidence that sublethal exposure to synthetic-based drilling fluids (SBDFs) induces neuromuscular toxicity in the brain of *Bunolagus monticularis*, as demonstrated by marked disruptions in electrolyte balance and selective alterations in electrolytes, and metabolic indices in exposed animals. These findings reinforce growing concerns regarding the neurotoxic and myotoxic potential of petroleum-derived drilling formulations, particularly in terrestrial vertebrates exposed through contaminated soil, forage, or water sources.

The brain electrolyte profile in Tables 1 and 2, shows marked alterations across exposure concentrations (0- 900 mg/L), indicating disruption of ionic homeostasis. $Na^{+}$ fluctuated significantly, with marked reduction at 300 and 700 mg/L, but elevation at 900 mg/L. $K^{+}$ increased at 300 mg/L, then progressively decreased at higher concentrations. $Cl^{-}$ elevated at 300 mg/L but reduced at 500 and 700 mg/L. $Mg^{2+}$ generally reduced across exposure groups compared to control.

Neuronal excitability depends primarily on $Na^{+}$ and $K^{+}$ gradients across membranes (Kandel et al., 2021). The disturbances suggest depolarization instability, altered repolarization, synaptic dysfunction, and inhibitory neurotransmission imbalance (Patani & Inyang, 2024). These alterations may manifest as impaired motor coordination, muscle weakness, tremors, or paralysis (Ogamba et al., 2011).

The observed fluctuations in brain sodium ($Na^{+}$) concentrations, characterized by significant elevations at higher exposure levels as presented in Table 1, are indicative of compromised neuronal ion regulation. Sodium homeostasis is critical for action potential initiation and synaptic transmission, and its dysregulation is a well-established marker of neurotoxic injury (Kandel et al., 2021). The biphasic pattern reported in this study suggests an initial compensatory response followed by failure of neuronal membrane integrity or $Na^{+}/K^{+}$-ATPase activity under sustained toxic stress. Similar exposure-dependent sodium perturbations have been documented in rodents and fish exposed to petroleum hydrocarbons, drilling mud additives, and organophosphate compounds (Inyang et al., 2010; Ogamba et al., 2011; Adeogun et al., 2022).

Potassium ($K^{+}$) depletion in brain tissue at higher SBDF concentrations further supports the presence of neurophysiological impairment. Potassium is indispensable for maintaining resting membrane potential and neurotransmission fidelity, and its reduction is commonly associated with neuronal hyperexcitability, muscle weakness, and impaired neuromuscular coordination (Guyton & Hall, 2020). Comparable potassium imbalances have been reported in rabbits exposed to Chlorpyrifos and other xenobiotics, where membrane permeability and ion channel dysfunction were implicated as primary mechanisms (Patani et al., 2020).

Alterations in chloride ($Cl^{-}$) levels observed in both brain and muscle tissues are particularly noteworthy, given chloride's role in inhibitory neurotransmission via GABAergic pathways. Declines in chloride availability can disrupt neuronal inhibition, promoting excitotoxicity and oxidative stress (Ben-Ari, 2017). Previous studies

on diazinon, Paraquat, and crude oil-derived contaminants similarly reported chloride imbalance as a contributor to neurobehavioral deficits and central nervous system stress (Ogamba et al., 2011; Inyang, 2008).

In contrast, magnesium ($Mg^{2+}$) concentrations remained statistically stable across treatments as shown in Tables 1 and 2. Magnesium serves as a natural calcium antagonist and stabilizer of neuronal membranes. Its stability may reflect efficient physiological buffering or lower affinity of SBDF components for magnesium-dependent regulatory pathways, consistent with selective electrolyte vulnerability following hydrocarbon exposure (Vincent-Akpu & Sikoki, 2013).

Skeletal muscle electrolyte levels in Table 2 showed significant concentration-dependent variations. $Na^+$ increased progressively at higher concentrations (700–900 mg/L). $K^+$ fluctuated but generally lower at higher doses. $Cl^-$ progressively declined, especially at 900 mg/L. $Mg^{2+}$ showed slight reductions at higher concentrations.

Muscle contraction depends on proper $Na^+$ influx, $K^+$ efflux, and $Mg^{2+}$ balance (Inyang et al., 2010). Disruptions suggest muscle hyperexcitability, impaired relaxation, reduced muscle stability, fatigue, cramps, and decreased contractile efficiency. These findings confirm interference with neuromuscular transmission and membrane stability (Kandel et al., 2021).

In skeletal muscle, the dose-dependent elevation in sodium concentration suggests disruption of sarcolemmal integrity and impaired transmembrane ion transport (Adeyemi et al., 2021). Such alterations are known to interfere with excitation–contraction coupling, potentially leading to muscle fatigue, weakness, and reduced locomotor efficiency (Allen et al., 2008). The concurrent decline in chloride levels further supports compromised muscle excitability and osmotic balance.

Similar muscle electrolyte disturbances have been reported in fish, amphibians, and mammals exposed to herbicides, pesticides, and petroleum-based effluents, where impaired contractility and structural degeneration of muscle fibers were observed (Ogamba et al., 2011; Adeyemi et al., 2021). The relative stability of potassium and magnesium levels in muscle tissue suggests that sodium and chloride ions may serve as sensitive early biomarkers of SBDF-induced muscular toxicity. The mechanism of neuromuscular toxicity likely involves membrane destabilization due to ionic imbalance, oxidative stress induced by drilling fluid components, disruption of $Na^+/K^+$-ATPase activity, impaired synaptic transmission, possible excitotoxicity due to altered $Mg^{2+}$ and $Cl^-$ regulation.

Tables 3 and 4 reveals the metabolic destabilization of exposed animals. Metabolic parameters provide insight into systemic and cellular toxicity. The results of this study reveals that total protein (TP) fluctuated, but remained relatively stable. Albumin (ALB) showed slight reductions at some doses. Urea remained relatively stable. Creatinine elevated at certain concentrations. Elevated creatinine may indicate muscle breakdown or altered energy metabolism, while changes in protein levels reflect cellular stress or mild neuroinflammation (Adeyemi et al., 2021). Increased creatinine correlates with muscle degeneration, reduced neuromuscular efficiency, and oxidative stress–induced tissue damage (Allen et al., 2008).

The lack of significant changes in total protein, albumin, and urea concentrations in exposed animal organs indicates that systemic protein metabolism and hepatic synthetic capacity were not critically compromised. This aligns with reports that sublethal drilling fluid exposure initially spares hepatic function while targeting excitable tissues (Vincent-Akpu & Sikoki, 2013; Adeogun et al., 2022). However, the significant reduction in muscle creatinine is toxicologically relevant. Creatinine reflects muscle energy turnover and renal handling, and its reduction may indicate suppressed muscle metabolism, enhanced renal clearance, or toxicant-induced muscle atrophy. Similar patterns have been reported in Chlorpyrifos and herbicide-exposed rabbits (Patani et al., 2020).

Despite being considered safer alternatives, synthetic drilling fluids pose significant neurotoxic and myotoxic risks. Chronic exposure in terrestrial ecosystems such as the Niger Delta may predispose wildlife and livestock to neuromuscular dysfunction before overt pathology. Electrolyte and metabolite perturbations represent early biomarkers of toxic stress and raise concerns regarding potential human health risks through bioaccumulation.

## V. Conclusion

The findings of this study therefore provide clear evidence that synthetic-based drilling fluids induce neuromuscular electrolyte imbalance and metabolic disruption in *Bunolagus monticularis*. While protein metabolism remains relatively stable, significant ionic dysregulation and reduced muscle creatinine highlight potential risks to neuromuscular and renal function. These findings underscore the need for strict regulatory oversight, environmentally safer drilling formulations, and continuous bio-monitoring in oil-producing regions such as the Niger Delta region of Nigeria.

**Ethical Issues**

The authors are all aware of ethical issues as stipulated by the laws of the land and thus completely complied with best practices while carrying out this research.

**Competing Interest**

Authors declare that there is no conflict of interest that would jeopardize the authenticity and originality of this scientific manuscript.

**Authors Contribution**

All authors of this research made equal contributions, ranging from data collection, analysis, manuscript proof reading and writing.

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