



(RESEARCH ARTICLE)



## Reproductive toxicity and biomarker response of male albino rats (*Rattus norvegicus*) exposed to cuprous oxide and petrol

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### Abstract

The effect of cuprous oxide and petrol on albino rats was studied using selected parameters associated with rat haematology and semen. The haematological parameters evaluated were Pack cell volume (PCV), Haemoglobin (Hb), Red blood cell (RBC), Mean corpuscular Volume (McV), Mean Corpuscular Haemoglobin (Mch) and Mean Corpuscular Haemoglobin Concentration (Mchc), while the semen analysis carried out includes; Motility quantitative (M1), Debris quantitative (D1), sperm count (C), morphology primordial (Mp) and morphology structure (Ms). The results revealed the following; Group 1, 2, 3 and 4 had a mean value of 43.0, 31.0, 26.75 and 23.75 respectively for PCV with significant difference ( $P < 0.05$ ) in all the treated groups when compared to the control group. (Hb) had a mean value of 13.65, 9.475, 9.50 and 11.075 for group 1, 2, 3 and 4 respectively with a significant difference ( $P < 0.05$ ) recorded in group 2 and 3 when compared to the control. Group 1, 2, 3 and 4 had a mean value of 4.968, 2.965, 1.63 and 1.815 respectively for red blood cell (RBC) with significant difference ( $P < 0.05$ ) across the groups. A mean value of 8.713, 10.453, 3.45 and 13.655 for group 1, 2, 3 and 4 respectively was recorded for McV with a significant difference ( $P < 0.05$ ) in group all treated group compared to the control. Group 1, 2, 3 and 4 had a mean value of 2.765, 3.450, 5.93 and 5.565 respectively for Mch. For Mchc, group 1 had a mean value of 0.315 group 2 had 0.305, group 3 had 0.355 and group 4 had 0.483 with a significance difference ( $P < 0.05$ ) recorded for only group 4. In the semen analysis, M1 had mean values of 68.75, 38.75, 28.0 and 27.5 in group 1, 2, 3 and 4 respectively, D1 had 7.5, 11.25, 24.5 and 42.5 in group 1, 2, 3 and 4 respectively, C had 65.0, 52.5, 29.0 and 42.0 in group 1, 2, 3 and 4 respectively while Mp had 7.5, 11.25, 7.5 and 22.5 in group 1, 2, 3 and 4 respectively. The results indicate that cuprous oxide and petrol both have negative effect on the body and hence care should be taken in the storage and handling of petrol to avoid human exposure, also the concentration of cuprous oxide to be used in antifouling paints should be regulated to avoid harming to aquatic organisms.

**Keywords:** Reproductive Toxicity; Haematology; Semen; Petrol; Cuprous oxide

### 1. Introduction

Biofouling is one of the main problems faced by ships on high sea. Marine growth such as barnacles and mussels have resulted in decreased ship efficiency, corrosion etc. Biofouling not only sticks to the external surface of the ships but also gets into the water intakes and sticks to the surface of the pipes leading to problems such as blockage and corrosion [1]. Antifouling agents are substances which prevent the growth and settlement of marine organisms on submerged structures such as ship bottom, oil rig supports, fish cages etc [2]. The use of these substances is the most important method in preventing fouling in modern maritime industries and boating communities. Different antifouling agents have been applied to prevent fouling of submerged structures. Biocides such as Tributyltin (TBT) compounds were the most active ingredients in the composition of antifouling agents and proved effective. However, the use of TBT

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compounds has been regulated internationally due to its toxic effects to both microorganisms and larger aquatic organisms [3]. Due to the adverse effect of TBT, different chemicals have been developed as antifouling biocides [4]. Copper has been used extensively as an antifoulant and studies have investigated to determine its bioavailability and toxicity which will help determine its fate in the environment (Thomas and Brooks, 2009). When these chemicals (biocide) leach from the paint into sea water, they get absorbed by marine organisms, persistent and bioaccumulative, and in the process remain in the environment and increase in concentration up the food chain. They have hormone-disrupting properties. Even at low concentrations it causes deformations in oysters and genital changes in snails [5]. A study carried out to assess the risk of antifoulants using fertilised cod eggs, the eggs were exposed to triazine, copper and TBTO singly or combined in laboratory tests with running seawater [5]. The result revealed that at the highest tested concentrations (11.5µg Cu l<sup>-1</sup>; 5µg TBTO l<sup>-1</sup>) larval mortality was increased. The highest concentration of triazine (40 µg l<sup>-1</sup>) did not cause any significant mortality. Fertilised eggs that had been exposed to all the three chemicals singly for five days showed a higher buoyancy than the controls. No synergistic or antagonistic effects were indicated. Embryos/larvae exposed to 0.004–0.8µg TBTO l<sup>-1</sup> did not show any changed respiration compared to the controls after hatching [5]. Petrol is a transparent liquid produced from the fractional distillation of crude oil and used as fuel in combustion engines. Composed of aliphatic and aromatic hydrocarbons, petrol is derived from blending fractions of crude oil with brand-specific additives [6]. The actual composition of petrol is dependent on the source of crude oil and the manufacturing process. Petroleum fumes are ubiquitous in the environment and poses significant risk of plant, animal and human health [7]. Petrol is known to be hazardous to human health and is associated with various health effects such as haematotoxicity and reproductive effects [7]. Pollution associated with petrol is well known, could be caused by natural or anthropogenic factors and can occur during production, transportation, storage, processing or use of the product [8]. Petrol has the tendency to elicit different types of toxic effects due to its complex composition. Human exposure to petrol may result in organ damage, although this is dependent on the duration of exposure and other demographic factors such as age, sex, etc., it is reported that mechanics and fuel attendants are more susceptible to health risk from petrol due to the nature of their job and constant exposure to petrol. Considering the foregoing, it is imperative that the adverse effect of petrol be investigated [9]. This study is aimed at evaluating the reproductive and haematological effects of cuprous oxide and petrol in exposed albino rats.

## 2. Material and methods

### 2.1. Experimental design

A total of twenty (20) healthy adult male albino rat (*Rattus norvegicus*) weighing 160 – 200 g were used for this experiment, and were allowed to acclimatize to laboratory condition (25°C) for 2 weeks. They were housed in a plastic cage during the duration of study. Complete Randomized design was used and the animals were divided into four different groups and carburettor cleaner solvent was used as the treatment; Group 1 (control, no chemical), Group 2 (0.1mg/kg body weight of cuprous oxide mixed with water orally), Group 3 (0.1mg/kg body weight of cuprous oxide incorporated in feed) and Group 4 (0.5ml of petrol orally).

### 2.2. Biochemical Analysis

Standard procedures were ensured during the collection of the blood, and sperm samples prior to biochemical analysis. Sperm fluid/semen was collected from the sperm duct by maceration on the glass slide and the analysis procedure was done according to [10], Sperm motility, viability and abnormalities were determined using one step eosin method [11] and the epididymal sperm count was done with Neubauer haemocytometer (Deep 1/10 mm, LABART, Munich, Germany) and light microscope at 40× magnifications.

### 2.3. Method of Data Analysis

Data were analyzed using Tukey test at a level of 5% probability, using Assitat Software Version 7.7 en (2017).

## 3. Results

### 3.1. Effects of Cuprous Oxide and Petrol on Semen analysis of Albino Rats

The results for semen analysis is outlined in Table 1, The control group (group 1) had a mean value of 66.725 for M1 (Motility quantitative), while group 2 had a mean value of 38.75, group 3 had a mean value of 28.0, group 4 had a mean value of 27.5. Statistically, there were significant differences (P<0.05) across the groups when comparing group 2, 3, and 4 with the control group (Group 1) and a significant difference (P<0.05) within group 2. For D1 (Debris quantitative), group 1, group 2 and group 3 had a mean value of 7.50, 11.25 and 24.5 respectively while group 4 had a

mean value of 42.5 with significant difference ( $P < 0.05$ ) recorded when comparing group 3 and group 4 with the control while group 1 had no significant difference ( $P > 0.05$ ) when compared to the control. The sperm count carried out (C) had a mean value of 65.0, 52.5 and 29.0 in group 1, group 2 and group 3 respectively, while it had a mean value of 42.5 in group 4. Group 2 was the only group that had no significant difference ( $P > 0.05$ ) when compared to the control. The Morphological premodial (Mp) had a mean value of 7.50 respectively in group 1 and group 3 while group 2 and group 4 had a mean value of 11.25 and 22.5 respectively. There was a significant difference ( $P < 0.05$ ) for group 2 and 4 when compared to the control.

### 3.2. Effects of Cuprous Oxide and Petrol on Haematological Parameters of Albino Rats

The results for Haematological parameters of Albino rats are shown in Table 2. Group 1, 2 and 3 had a mean value of 43.0, 31.0 and 26.75 respectively for Pack cell volume (PCV) and group 4 had a mean value of 23.75 for PCV with significant difference ( $P < 0.05$ ) in all the treated groups when compared to the control group and group 2 had a significant difference ( $P < 0.05$ ) within the group. Haemoglobin (Hb) had a mean value of 13.65, 9.475, 9.50 and 11.075 for group 1, group 2, group 3 and group 4 respectively with a significant difference ( $P < 0.05$ ) recorded in group 2 and 3 when compared to the control. Group 1, 2, 3 and 4 had a mean value of 4.968, 2.965, 1.63 and 1.815 respectively for red blood cell (RBC) with significant difference ( $P < 0.05$ ) across the groups. Mean corpuscular Volume (McV) had mean value of 8.713, 10.453, 3.45 and 13.655 for group 1, group 2, group 3 and group 4 respectively with a significant difference ( $P < 0.05$ ) in group all treated group compared to the control. Group 1, 2, 3 and 4 had a mean value of 2.765, 3.450, 5.93 and 5.565 respectively for McH (Mean Corpuscular Haemoglobin). Statistically, there was significant difference ( $P < 0.05$ ) in group 2, 3 and 4 when compared to the control group. For McHc (Mean Corpuscular Haemoglobin Concentration), group 1 had a mean value of 0.315 group 2 had 0.305, group 3 had 0.355 and group 4 had 0.483 with a significance difference ( $P < 0.05$ ) recorded for only group 4.

**Table 1** Semen Analysis

	M1	D1	C	Mp
Group 1	68.75±10.3 <sup>a</sup>	7.50±2.89 <sup>c</sup>	65.00±6.68 <sup>a</sup>	7.50±2.87 <sup>c</sup>
Group 2	38.75±6.29 <sup>b,c</sup>	11.25±4.79 <sup>c</sup>	52.50±8.89 <sup>a</sup>	11.25±7.50 <sup>b</sup>
Group 3	28.00±2.45 <sup>c</sup>	24.5±8.81 <sup>b</sup>	29.00±2.16 <sup>c</sup>	7.50±5.00 <sup>c</sup>
Group 4	27.5±6.45 <sup>c</sup>	42.5±6.45 <sup>a</sup>	42.0±8.83 <sup>c</sup>	22.5±13.23 <sup>a</sup>

**Key:** Motility quantitative (M1), Debris quantitative (D1), sperm count (C), and morphology primordial (Mp); <sup>A-B</sup>Different letters in the same column indicate significant difference ( $P < 0.05$ ) within the group; <sup>a-b</sup> Different letters in the same column indicate significant difference ( $P < 0.05$ ) across the group

**Table 2** Hematological Analysis

	PCV	HB	RBC	McV	MCH	MCHc
Group 1	43.00±5.099 <sup>a</sup>	13.65±1.126 <sup>a</sup>	4.968±0.617 <sup>a</sup>	8.713±1.021 <sup>b</sup>	2.765±0.255 <sup>b</sup>	0.315±0.017 <sup>b</sup>
Group 2	31.00±3.367 <sup>a,B</sup>	9.475±1.634 <sup>b</sup>	2.965±0.034 <sup>b</sup>	10.453±1.082 <sup>aB</sup>	3.450±0.466 <sup>a,B</sup>	0.305±0.05 <sup>b</sup>
Group 3	26.75±2.363 <sup>b</sup>	9.50±1.257 <sup>b</sup>	1.63±0.179 <sup>c</sup>	3.45±0.466 <sup>c</sup>	5.93±1.326 <sup>a</sup>	0.355±0.024 <sup>b</sup>
Group 4	23.75±5.0 <sup>b</sup>	11.075±1.33 <sup>a</sup>	1.815±0.474 <sup>b,c</sup>	13.655±4.226 <sup>a</sup>	5.565±1.631 <sup>a</sup>	0.483±0.100 <sup>a</sup>

**Key:** Pack cell volume (PCV), Haemoglobin (Hb), Red blood cell (RBC), Mean corpuscular Volume (McV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHc); <sup>a-b</sup> Different letters in the same column indicate significant difference ( $P < 0.05$ ) within the group; <sup>A-B</sup> Different letters in the same column indicate significant difference ( $P < 0.05$ ) across the group

## 4. Discussion

The results in Table 1 indicate that both cuprous oxide and petrol both had a negative effect on the semen generally. The sperm motility reduced significantly in the treated group, with the lowest recorded in group 4 with is treated with petrol, there was also marked negative effect on the sperm count and morphology with an increase in debris quantitative. There has not been any reported direct effect of petrol or cuprous oxide on sperm production, but these negative effects observed might be due to the negative effect exerted on the body as seen in the haematological analysis. According to [12], anything that can affects the normal functioning of the hypothalamus and the pituitary glands can affect sperm production since they produce hormones that play important role in sperm production. Excess intake of

copper causes copper toxicosis which is known to affect the central nervous system alongside other organs and system [13 & 14] and since the hypothalamus and pituitary glands are attached to the brain which is part of the central nervous system, one can therefore say that cuprous oxide which releases copper during its breakdown can cause copper toxicosis which will possibly affect the brain and then affect the hypothalamus and pituitary glands which in turn will affect the sperm production. The haematological parameters indicate that the treatments affected the body negatively. The PCV was significantly lower in the treated group and this indicates the development of an anaemic condition, this is in agreement with [15] that a suppressive effect of petroleum products on erythropoiesis. Reduction in the value of PCV and Hb has also been attributed to the inducement of bone hypoplasia by petroleum products [16 & 17]. These adverse effects of petrol on blood have been attributed to possible presence of toxic components like Xylene and benzene in petroleum products like petrol [18]. The results generally are in agreement with haematotoxic reports of petrol by different authors [7, 8, 19, & 20]. Exposure to copper via oral ingestion according to [21] can cause liver and kidney damage. This explains why there were deleterious alterations in the blood parameters in group 2 and 3. Any damages done to the kidney can also lead to the decrease in the value of PCV considering that the kidney plays a vital role in regulating the PCV [22]. Damages done to the kidney (leading to kidney diseases) also affects the red blood cell negatively by reducing the amount of red blood cell in circulation, this might be why the red blood cell analyzed was extremely low, the kidney is known to play a vital role in red blood cell production via erythropoietin hormone secreted by the peritubular capillary lining cells of the kidney [23]. High McV especially for group 2 and 4 indicates macrocytic condition of the blood [24], this can be as a result of the general low amount of red blood cells as seen in table 2 which must have caused the few red blood cells produced to be larger in size in a bid to meet up with the demands of oxygen by the body. This is based on the biological phenomenon called physiological hypertrophy, although this is usually common among muscle cells. McH was generally high in the treated groups compared to the control group is a sign of macrocytic anaemia and is known to arise due to liver damage.

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## 5. Conclusion

Standardization in the concentration of Cuprous oxide to be used should be done and carefully monitored because although useful as an antifouling agent, it has a negative effect on life while Petrol should always be handled with care and stored away from foods and water to avoid food poisoning with the accidental consumption of such contaminated food and water.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

No potential conflict of interest reported by the authors.

### *Statement of ethical approval*

University standard written ethical permission was sought for, granted and has been preserved by the author(s).

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