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Assessment of Water Stored in Washed Lubricating Oil Containers and Its Effects on Kidney and Blood Functions in Albino Wistar Rats

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Abstract
The aim of this study is to evaluate effects of water stored in washed lubricating oil container (engine oil) on the kidney and blood function of an albino Wistar rat. Four sampling groups were established, group1 was control while three other groups were experimental groups. Total of twenty four (24) matured albino Wistar rats were investigated and acclimatized for seven (7) days, before rats were grouped into four of six rats each in the groups. The first group (control) was placed on control water as source of drinking water for 21days while other groups (test) was placed on water stored in washed lubricating oil container (engine oil) for a period of 28 days. The rats were fed on commercial feeds, at the end of the experiment the rats were weighed and sacrificed by anaesthetizing in a container containing cotton wool soaked in chloroform. Samples collected were kidney and blood. ANOVA was used to calculate mean and standard deviation while statistical significance was assessed at 0.05 probability level using Tukey-tests($p<0.05$). kidney and lipid function parameters investigated were urea, creatinine, potassium, sodium, chloride, bicarbonate, total cholesterol, triglyceride, high-density lipoprotein and low-density lipoprotein, followed by heamatological test, which involves PCV, Hb, RBC, WBC, Platelet, MCHC, MCH, MCV, Neutrophil, Lymphocyte, Eosinophil and monocyte. Slight increase was observed in most of the results while in blood test, little decrease was noticed in group2. So water stored in washed lubricating oil container should be discouraged for drinking, because drinking it for a long period may pose future risky to human health.

Keywords: Effects; Water; Albino Wistar Rat; Lubricating oil; Blood; Kidney

Introduction

Water cannot be separated from the daily life activities of human beings and animals due to its importance to human life and animals. Water is said to be an aspect or part of living organisms including humans. Water is needed at home, in agricultural activities, industries, etc. . So many people have access to water, and also give their animals water to drink daily, but due to poverty or ignorant some people like to buy containers of spent engine oil or lubricating oil and washed it for storage of drinking water either by themselves or their animals, sometime it is used for cooking food and bathing specially people in the rural areas, without considering the side effect, even when the oil is giving taste to the water and this may be source of many sickness and diseases (Ekeh et al., 2010).. In this regard, this study was carefully carried out to assess physiochemical properties of water stored in washed lubricating oil container and its effects on the kidney and blood function of an albino wistar rat. In addition the disposal of water from lubricating oil into open vacant plots and gutters, farms and water drains is an environmental risk considering the water table in some part of Nigeria specially South-South Region of the country and shallow bore-holes dug to get water for domestic use (Egwurugwu et al., 2013). The presence of oil in the soil makes the soil condition unsatisfactory for plant growth due to decrease in the level of plants nutrient availability or increase in toxic level of certain elements such as iron and zinc (Maity et al, 2008).

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The rise in population has detrimental impacts on aquatic environments. This is as a result of industrial input sources which directly discharged different types of chemicals through effluents or wastewater to aquatic systems (Chauhan, 2014). It is an established fact that these chemical constituents discharged into the environment have negative environmental effects on aquatic biota and humans (Dhingra et al., 2015). One of the ways through which pollutants or contaminants are introduced by industries into the aquatic environment or water bodies is by wastewater discharge (Singh et al., 2012). These pollutants contribute to the level of oxygen available in the water and also the nutrient content potentials of the water bodies, thus promoting poisonous environment and leading to an unstable aquatic ecosystem (Morrison et al, 2001). Ever increasing quantity of wastes from industries and private homes are practically discharged into surface waters. In some or most cases, the treatment provided by the effluent discharging industry may not be adequate to provide the required level of protection that the receiving water body of environment requires (Edori & Nna, 2018)**.**

There are a lot of clearing products available in the market and majority are referred as detergent. Detergent is a substance or mixture containing soap surfactants (any organic mixture or substance) intended for washing and clearing process(Ubani et al.,2012). A detergent may be in many forms such as traditional powers, unit dose tablet, concentrated liquids, capsules and paste (Elechi & Alikor 2017). In this research a particular detergent chosen to wish the lubricating oil container is called **"viva plus"** this particular detergent is made up of sodium carbonate, sodium silicate, sodium sulphate, polycarboxylate, vinylpyroliodone, vinylimidazole, enzymes and perfume, which many contribute to the available result in this project, and the detergent is in powder form. Detergents are substances when dissolved in water possess the ability to remove dirt from surfaces such as the human skin, textiles and other solids (Singh et al., 2012).

Oil storage containers should have spaces for properly label the type of oil inside and to comply with the Occupational Safety and Health Administration's Right to known federal law. This law states that employees have the right to know what chemicals they're working with, so labels must show the product inside the storage container, who makes it and note any dangers the substance holds (Agoro et al., 2019).

In this study the lubricating oil container used are;

1. Techno HD40, product of techno oil limited, the container is yellow in colour, it has different sizes which ranged from 1liter to 10 liters, in this work 5litres was used, it is a plastic container.
2. Mobil 1 5w-30, product of Exxonmobil corp, the plastic container has silver colour and the container is 5liters in size.
3. Nippon runner 5w30 motor oil, a product of Nippon Company limited, the container is 5 liters in size with bronze colour.

Lubricating oils may be either mineral-based or synthetic. This work only focused on petroleum-based lubricating oils, which composed of 80–90% of petroleum hydrocarbon distillates and 10–20% of additives and are used for lubrication of various internal combustion engines. Occupational and general populations are exposed to lubricating oil mainly through inhalation and dermal contact. Acute exposure has been associated with irritation of the eyes, skin, and respiratory tract infection (Ubani & Joshua, 2010).
Rats are usually supplied feed free choice and they eat 10-30g a day (5g/100g body weight/day). Water is supplied free choice and they usually drink 20-50ml a day (10 ml/100g body weight/day). Water may be supplied using a bottle or automatic waters, and may be further treated by reverse osmosis, ozone, ultraviolet radiation, hyperchlorination or acidification. Rats were first used for experimental purposes in the mid1800s. Strains were developed to study neuroanatomy, nutrition, endocrinology, genetics and behavior (Karimi et al 2012).

Materials and Method

Study Area

The study was carried out in the Research Laboratory of Biochemistry Department University of Port Harcourt in Port Harcourt, Rivers State Nigeria. The experiment was conducted between September and October 2024.

Source of Experimental Rat

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Total of twenty four (24) matured albino Wistar rats were used, they were obtained from the animal house of the Department of Biochemistry, University of Port Harcourt. They were housed in clean and well ventilated polypropylene cages supplied of food and water from the washed container of lubricating oil throughout the experiment and the control were served with normal water.

Collection of Test Water

Lubricating oil containers was collected from various mechanic workshops in Obio- Akpor Local Government Area of Rivers State Nigeria. Washed thoroughly with viva plus detergent and filled with water.

Acclimation of rat

Twenty four (24) adult Wistar albino rats that weighed between 76g to 106g before they were acclimatized for seven (7) days, the rats were assigned to four groups comprising of six animals each.

Experimental Protocol

The experimental animals were arranged into four groups, each group comprising six animals.

Group 1: Normal control rats (feed only and clean water) for 21days

Group 2: Normal feed and water from washed lubricating oil container for 21days (Techno engine oil container).

Group 3: Normal feed and water from washed lubricating oil container for 21 days (Mobil 1 engine oil container).

Group 4: Normal feed and water from washed lubricating oil container for 21 days (Nippon Runner engine oil container).

Laboratory Analysis

Histopathological Analysis (Kidney function test)

The follow below were assessed in the kidney

Urea (Ur)

Urea in serum was hydrolyzed to ammonia in the presence of urease. The ammonia was measured photometrically by Berthelot's reaction. There are three different test tubes labeled sample, blank and standard, 0.1ml of the reagent (R_1) was pipetted into all the test tubes, 10µl of the samples, standard and distilled water was added into the appropriate tubes mixed and incubated at 37^0c for 10minutes, 2.5ml of R_2 & R_3 was pipetted to all the tubes mixed and incubated at 25ºc for 15minutes and the absorbance was read and recorded at 546nm.

Creatinine (Cr)

Creatinine reacted with picric acid in alkaline solution to form a coloured complex. The amount of complex formed is directly proportional to the creatinnine concentration. The test tubes were labeled sample, blank and standard, 2.0ml of the reagent (R_1) was pipetted into all the test tubes, and 0.1ml of the sample, standard and distilled water was added into respective tubes, mixed carefully and after 30 minutes, the absorbance and of the standard and was calculated .

Potassium (K)

Sodium tretraphenylboron was used to determine the amount of potassium in a specially prepared mixture to produce a colloidal suspension. The turbidity of which is proportional potassium concentration in the sample. The test tubes were labeled sample, standard and blank. 1ml of the reagent was pipetted into all the test tubes. 10ul of the samples added into appropriate tubes mixed and allowed for 3 minutes at 25ºC. The spectrophotometer was zeroed using blank at 500nm and absorbance was read and recorded.

Sodium (Na)

The method used here was based on reaction of sodium with a selective chromogen producing a chromophore whose absorbance is directly as the concentration of sodium in the sample. The test tubes was labeled sample, standard and blank. 0.01ml of the reagent was pipetted into all the test tubes. 0.01ml of the sample was added into appropriate tubes mixed and incubated for 5 minutes at 25ºC and absorbance was read and recorded at 630nm.

Chloride (CL)

The quantitative displacement of thiocyanate by chloride from mercuric thiocyanate any subsequent formation of a red ferric thiocyanate complex was measured calorimetrically. The test tubes were labeled sample, blank and standard. 1.0ml of the reagent was pipetted into all the test tube. 10ul of the sample was added into appropriate tubes mixed and incubated for 5 minutes at 25ºC and absorbance was read and recorded at 480nm.

Bicarbonate ($HC0_3$)

Serum HCo_3 was reacted with excess standard HCL . The remaining HCL was back titrated with standard NoaH using phenol red as indicator. Co_2-free distilled water was added into 250ul 50ml conical flask, 200ul sample, 0.01N, HCL 1ml, mixed well and added 3 drops of phenol red, the flask was whirl to release the $C0_2$. The resultant

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solution was titrated with 0.01N and NaoH until the initial light yellow colour fades to a light purple at the end point. The remaining NaoH that does not take part in the reaction was read. The reading obtained was divided by two; this gave the concentration of HCo_3 in the sample (unit mmol/L).

Cholesterol

The cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine was formed from hydrogen peroxide and 4- aminoantipyrine in the presence of phenol and peroxidase.

The test tubes were labeled sample, blank and standard, 1.0ml of the reagent was pipetted into all the test tubes. 10µl of the sample was added into appropriate tubes mixed and incubated for 5 minutes at 25^{o}C and absorbance was read and recorded at 540nm.

Triglycerides

The triglycerides was determined after enzymatic hydrolysis with lipases. The indicator is quinoneimine formed from hydrogen-peroxide, 4 – aminophenazone and 4 – chlorophenol under the catayltic influence of peroxidase.

The test tubes were labeled sample, blank and standard, 1.0ml of the reagent was pipetted into all the tubes. 10ul of the sample was added into appropriate tubes mixed and incubated for 5 minutes at 25^{o}C and absorbance was read and recorded at 540nm.

High Density Lipoprotein – Cholesterol (HDL)

Low density lipoprotein, VLDL and Chylomicron fractions was precipitated by the addition of phosphotungestic acid in the presence of magnesium ions. After centrifugation the cholesterol concentration in the HDL fraction which remains in the supernatant was determined. The test tubes were labeled sample, blank and standard, 0.7ml of the reagent was pipetted into all the test tubes, 40ul of the sample was added into appropriate tubes mixed and centrifuge for 10mins at 4000rpm.

Low Density Lipoprotein – Cholesterol (LDL)

Low density lipoprotein, VLDL and Chylomicron fractions was precipitated by the addition of phosphotungestic acid in the presence of magnesium ions. After centrifugation the cholesterol concentration in the HDL fraction which remains in the supernatant was determined. The test tubes were labeled sample, blank and standard, 01.0ml of chlorine reagent was pipetted into all the test tubes, 40ul of supernatant was added into appropriate tubes mixed and incubated for 5minuts at 25^{o}c, and absorbance was read and recorded at 540nm.

Haematological Analysis

The following below was analyzed haematologically

packed cell volume (PCV); The packed cell volume is that proportion of whole blood occupied by red blood cells expressed as a ratio (L/L). ¾ of the capillary tube was filled with well-mixed EDTA blood, sealed with unfilled end place in microhaematocrit rotor, and centrifuge for 5 minutes immediately after centrifuging; PCV was read using the microhaematorcrit reader.

Haemoglobin (Hb) **;** For Haemoglobin, PCV value was divided by 3 Hb unit: mg/dl, in other to get accurate Hb .

Red blood cell count (RBC); 4.0ml of formal citrate (diluting fluid) was measured and dispensed into a test tube; 0.02ml of well-mixed EDTA blood was added and mixed. Counting chamber was assembled and filled with well-mixed sample, the chamber was left undisturbed after that it was examined using x10 objective lens and red cells was counted in the small squares and the number of red cells was read per liter.

White blood cell (WBC)

0.38ml of diluting fluid was pipetted into the test tubes, 0.02ml of well-mixed EDTA blood was added and mixed and counting chamber was assembled and remixed with diluted blood sample using Pasteur pipette filled with one of the grids of the chamber with sample. The chamber was left undisturbed for 20 minutes to allow time for the white cells to settle, X10 objective lens used was examined and the cells was counted in four large squares of the chamber. The number of white cells per liter was reported.

Neutrophil, lymphocyte, eosinophil, and monocyte; Was screened using automated haematology analyzers count.

Platelet (PLT) 0.38ml of diluting fluid was pipetted into the test tubes, 0.02ml of well-mixed EDTA blood was added and mixed and counting chamber was assembled and remixed with diluted blood sample using Pasteur pipette filled with one of the grids of the chamber with sample. The chamber was left undisturbed for 20 minutes to prevent drying of the fluid placed on the chamber in a Petri dish on dampened tissue and covered with a lid, X10 objective

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lens used was examined and the platelet was counted in four large squares of the chamber. The number of the platelet per liter was reported.

Platelet count $\frac{N \times 20 \times 106}{0.2 \times 0.1}$

In producing a chromophore whose absorbance varies directly as the concentration of sodium in the test sample. The test tubes were labeled sample, blank, and standard, 1.0ml of the reagent was pipetted into all the test tubes, 0.01ml of the sample was added into appropriate tubes mixed, and incubated for 5 minutes at 25°C, and absorbance was read and recorded at 630nm.

Data Analysis

The results of the samples were analyzed using origin-pro version 9.9.0.225 package. Descriptive statistics of variance (ANOVA) was used; statistical significance was assessed at 0.05 probability level using Tukey tests. The means of the treatments was repeated using least significant difference (LSD) test.

Results

Table.1 Showing the results of the kidney function parameters of the test and control rat

Parameters	**Group**			
	GROUP1	GROUP2	GROUP3	GROUP4
Ur(mmo/l)	^{B}2.917±0.574	^{B}3.420±0.476	^{B}3.400±0.316	^{A}4.200±0.410
Cr(mmo/l)	^{B}62.67±7.28	^{B}70.60±8.96	^{B}71.50±7.56	^{A} 87.67±7.42
K(mmo/l)	^{A}3.183±0.581	^{A}3.560±0.344	^{A}10.47±16.92	^{A}3.200±0.322
Na(mmo/l)	^{A}116.33±17.15	^{A}132.80±8.17	^{A}134.33±11.31	^{A}121.83±8.13
CL(mmo/l)	^{A}59.17±7.68	^{A}60.40±2.70	^{A}64.83±5.74	^{A} 60.167±2.041
HCO_3(mmo/l)	^{B}22.667±1.633	^{A}28.800±1.3	^{AB}25.17±3.31	^{B}24.00±2.53
TC(mmo/l)	^{A}3.200±0.502	^{A}3.600±0.474	^{A}3.600±0.477	^{A}3.483±0.454
TG(mmo/l)	^{A}1.168±0.263	^{A}1.176±0.128	^{A}1.287±0.209	^{A}3.42±5.58
HDL(mg/dl)	^{A}1.493±0.218	^{A}1.472±0.162	^{A}1.548±0.162	^{A}1.453±0.218
LDL(mg/dl)	^{A}2.238±0.487	^{A}2.464±0.698	^{A}2.635±0.448	^{A}2.397±0.243

Superscripts with the same alphabet are not significantly different (Tukey-tests, p<0.05)

Table.2 Showing the results of the blood function parameters of the test and control rat

Parameters	**Group**			
	GROUP1	GROUP2	GROUP3	GROUP4
PCV((%))	^{A}41.00±1.549	^{AB}40.17±4.22	^{AB}39.00±3.35	^{B}35.17±4.12
Hb(g/dL)	^{A}13.433±0.320	^{A}13.083±1.158	^{A}12.183±1.411	^{A}12.133±0.924
RBC(m/mm^3)	^{A}7.150±0.187	^{AB}6.733±0.606	^{A}7.250±0.883	^{B}5.950±0.547
WBC(m/mm^3)	^{A}9.65±6.72	^{A}6.617±1.786	^{A}8.42±3.81	^{A}10.45±4.59
PLT(femotolitre)	^{A}540±267	^{A}526.0±182.6	^{A}537.7±195.6	^{A}402.0±160.4
MCHC	^{B}32.367±1.214	^{B}32.550±1.883	^{AB}31.233±1.908	^{A}34.100±1.409
MCH(Pg/Cell)	^{AB}18.683±0.591	^{A}19.350±0.812	^{B}16.833±2.140	^{A}20.333±1.155
MCV(%)	^{AB}57.733±1.394	^{A}59.700±1.667	^{B}53.87±4.62	^{AB}59.12±4.64
Neutrophil(N)(%)	^{A}10.00±5.51	^{A}8.50±5.75	^{A}7.833±1.169	^{A}9.83±7.08
Lymphocyte (L) (Microlitre)	^{A}83.17±7.81	^{A}85.00±7.75	^{A}85.17±2.64	^{A}82.00±11.40
Eosinophil (E) (Cell/McL)	^{A}1.167±0.408	^{A}1.333±0.516	^{A}1.167±0.408	^{A}1.167±0.407
Monocyte (M)(%)	^{B}5.667±2.422	^{B}5.167±1.941	^{B}5.833±1.941	^{A}7.00±4.15

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Superscripts with the same alphabet are not significantly different (Tukey-tests, $p<0.05$)

Discussion

Urea is commonly used to assess renal function, it is a nitrogenous waste product made from breakdown of proteins cleared from serum and excreted by the kidney. The normal level of urea ranged between 2.5 to 7.8mmol/l(Babalola, & Oni, 2018). In this study, urea means value ranged between 2.917±0.574 to 4.200±0.410mmol/l which showed normal levels of urea, when compared with control result, there was increase within the sampled groups

Creatinine is a product of skeletal muscle breakdown cleared from serum and excreted by the kidney. Normal levels of creatinine ranged between 60 to 105µmol/L(Arise et al., 2012). The mean value of creatinine ranged between 62.67±7.28 to 87.67±7.42 µmol/L which shows normal levels of creatinine, but the experimental groups has higher levels of creatinine when compared with control result, which may be as a result of water drank by the rats.

Potassium is therefore critical for propagation of action potentials for muscle contraction and neurotransmitter release. Small changes in potassium can therefore affect cardiac rhythm. It is also important in acid-base balance. The normal levels of Potassium ranged between 3.5 – 5.3mmol/L(APHA., 2012). The result of this study shows that potassium mean value ranged between 3.183±0.581 to 10.47±16.92mmol/L, the results shows that potassium levels were normal except group three, which was above the normal level of potassium. Group three water was stored with Mobil 1 engine oil container which has some smell after washing it, which may be as a result of certain products or chemicals used in manufacturing the container. The result of sodium (Na), chloride (CL) and Bicarbonate (HCO_3) exposed increase in levels when compared with the control, which means continuous drinking of the water stored in lubricating oil containers for a longer period of time, can increase the concentrations of sodium, chloride and Bicarbonate above the normal level.

Total cholesterol is an unsaturated alcohol of the steroid family of compounds; it is essential for the normal function of all animal cells and is a fundamental element of their cell membranes. It is also a precursor of various critical substances such as adrenal and gonadal steroid hormones and bile acids. It has normal level range of 3 to 5.5mmol/l (Alimba et al., 2012). The result of this study shows that total cholesterol (T.C) mean value ranged between 3.200±0.502 to 3.600±0.477mmol/L, when compared with control result; it shows that there is little increase within the sampled groups.

Triglycerides (TG), High-density lipoprotein (HDL) and Low-density lipoprotein (LPL) shows that there is increase within the sampled groups when compared with control result.

Packed cell volume (PCV) is a measurement of the proportion of blood that is made up of cells; the value is expressed as a percentage or fraction of cells in blood. There is decrease in PCV when compared with control result, which demonstrates the fact that the water stored in the lubricating oil containers causes a significant reduction in the haematological parameters. Heamoglobin (Hb) is a protein in the red blood cells that carries oxygen to the body's organs, tissues and transports carbon dioxide from the organs and tissues back to the lungs. Heamoglobin test measures the amount of hemoglobin in your blood and normal levels of heamoglobin range between 12 to 18g/dl (Adeyemi & Adebayo 2009). The mean value of heamoglobin ranged between 12.133±0.924 to 13.433±0.320g/dl in this study, the result showed normal levels of heamoglobin, when compared with control result; it shows that there is little decrease within the sampled groups. Red blood cells (RBC) mean value shows decrease in normal levels when compared with control result.

A normal white blood cell count indicates that immune system is functioning as normal. The normal range for a white blood cell count is between 4-10X10^9L(Baratta et al., 2009). In this study white blood cell (WBC) and Platelet shows that there are little decrease when compared with control.

Mean Corpuscular Haemoglobin Concentrations (MCHC) indicates the amount of heamoglobin per unit volume and its normal range is between 30-35g/dl (Abdel et a., 2010l). MCHC and corpuscular hemoglobin (MCH) shows that

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there is little decrease when compared with control result. Neutrophil, Lymphocyte and Eosinophil shows decrease within the sampled groups, when compared with control result.

Conclusion

Some kidney and lipid function parameters investigated were urea, creatinine, potassium, sodium, chloride, bicarbonate, total cholesterol, triglyceride, high-density lipoprotein and low-density lipoprotein, followed by heamatological test, which involves PCV, Hb, RBC, WBC, Platelet, MCHC, MCH, MCV, Neutrophil, Lymphocyte, Eosinophil and monocyte. All the parameters investigated were compared with normal levels, and they were within permissible limit, when compared with control results some decreases were noticed while in some groups increase was observed. So water stored in washed lubricating oil container should be taken with caution and other detergents can still be used to wash the lubricating oil container for further investigation.

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