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Original Article

Consequences of long-term consumption of water from Nworie River (Owerri, Nigeria) on haematological, hepatic, and renal functions using rat model

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ABSTRACT: The consequences of long term consumption of water from Nworie River (Owerri, Nigeria) on haematological, hepatic, and renal functions using rat model (Wistar albino strain). Twenty-four rats separated into two groups of twelve rats each were kept as test and control for sixty-four days. The test rats were placed on water from Nworie River while those of the control were placed on Eva water (purified Coca-cola bottled water). The rats were sacrificed in two sets: first set was on thirty-second day while the second set was on the sixty-fourth day. Six rats each from each group were sacrificed at each set. The Results obtained revealed that Hb, PCV, WBC, L, N, and ESR were significantly affected ($p < 0.05$) in test rats against the control rats. The functional parameters of liver adequacy; AST, ALT, and ALP were significantly ($p < 0.05$) affected in test rats against those of the control. Also, urea and electrolyte ions (Potassium ion, chloride and bicarbonate) indicating renal sufficiency were significantly ($p < 0.05$) affected in test rats against those of the control. Creatinine, sodium ion, total bilirubin and conjugated bilirubin were not significantly affected ($p > 0.05$) in test rats when compared to those of the control. The induced changes in the parameters investigated in this study have shown that long-term consumption of water from Nworie River has effect on haematological, hepatic, and renal function.

KEYWORDS: *Nworie River, haematology, liver function, kidney function*

INTRODUCTION

The problem of accessibility and availability of clean water in developing countries is on the increase (Adekunle, 2004; Odeyemi *et al*; 2010). Rapid urbanization, domestic and industrial activities have greatly contributed to increased pollution of water bodies (Akubugwo and Duru, 2011; Nwigwe and Okpi, 2002). Pollution has reduced the portable nature of water from water bodies. This has also necessitated that water from these natural water bodies be further purified for domestic applications. It has been speculated that as long as the high population growth rate coupled with lack of improved sanitation exist in developing countries, water bodies will continue to be polluted. Environmental managers, water resources analysts, hydrologists, and allied professionals have been charged to fashion out ways to curb pollution and its evil effect to environment and natural water bodies (Ibeh and Mbah, 2007).

Results from previous studies have shown a direct link between polluted water and public health in terms of water-related diseases such as cholera, typhoid, diarrhoea, etc. Thousands of deaths have also been linked to incidence of water borne diseases in the rural and urban areas of most developing countries (UNO, 1983; World Bank, 1993; Haddinott, 1997; Brockerhoff, 1995). UNEP (1993) traced some of these deaths to the use of water grossly polluted through human activities.

Nworie River, one of the major rivers that flow through Owerri, the capital city of Imo State, Nigeria, is among those natural water bodies polluted through human activities. Nworie River is located between latitude 5º28N and 5º31N (Atlas Map). It has its major accessible sites within Owerri municipal. Owerri municipal has an estimated 125,337 people within it, and houses the Imo state capital (NPC, 2006). There is no doubt that increased economic activities have attracted people from other parts of the state and the nation into the municipality hence, making them more in number than the indigenes. Most indigenes of Owerri municipal depend on the river water for their domestic activities such as cooking, bathing, washing, etc, whereas the failure on the part of the public water supply authority (popularly known as "Water Board" within the municipality) drives most non-indigenes to depend on the river water as well.

Wastes generated as a result of human activities from institutions such as Federal Medical Centre (FMC), Alvan Ikoku Federal College of Education (AIFCE), and Holy Ghost College in Owerri, situated along the river banks, as well as wastes from most hotels situated within the municipality, do find their ways into the river, thereby polluting it. Washing, bathing, and other human activities carried out at different points to the river serve as additional sources of pollutants to it. Agrochemicals from farmlands surrounding the river get into it during rainfall. As a natural water body, it supplies water and fishing grounds to the local population.

Due to the importance of this river as a source of water especially when public water supply fails, and as part of our on-going research on the river, the present study investigated the consequences of long-term consumption of Nworie river on the haematological, hepatic, and renal functions using rat model.

MATERIALS AND METHODS

Collection of water from Nworie River

The water given to experimental animals was collected as composite samples at different points along Nworie River in clean plastic container.

Experimental animals

A total of twenty-four stocks of albino rats (Wister strain) were obtained from the animal colony of the Department of Biochemistry, Abia State University, Uturu, Abia State, Nigeria. The rats were weighed and allocated to two groups of twelve rats each. The groups were equalized as nearly as possible with respect to body weight. The rats were housed in plastic cages covered with wire meshes and had facilities for food and water in them. The rats were given the same feed and water for the initial acclimatization period of three days before administration commenced. Pelletized commercial rat feed (Pfizer Livestock Co. Ltd, Aba, Nigeria) was the rat feed. After acclimatization period, the test group was given water from Nworie River, while the control group was given Eva (Cola-cola product) water. All animals were treated according to NHSC instructions while the feeding lasted. The feeding lasted for a period of sixty-four days (Two months and four days). The rats were sacrificed in two sets. The first set was on the thirty-second day (after one month), and the second set on the sixty-fourth day (after two months). Twelve rats, six each from both groups were sacrificed during each set of sacrifice. The rats were sacrificed by making incisions at their cervical regions with sterile blades after being put to sleep (euthanization) in a close container with the help of chloroform. Blood samples for kidney and liver function analysis were collected into anticoagulant-free tubes with corks, while the one for haematology test was collected into heparin-treated tubes with corks. The tubes were appropriately labelled and were subsequently used for analysis.

Serum assay

The level of alkaline phosphatase (ALP) was determined by the method of Wright *et al* (1972). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined as described by Reitman and Frankel (1957). The assay of bilirubin both total and conjugated was carried out using the Jendrasik and Groff (1938). Creatinine was determined as described by Heinegard and Tiderstorm (1973). Urea estimation was carried out using Urease-Berthlot method. Potassium ion was determined by direct spectrometric method. Sodium ion level was detected using the methods described by Maruna (1958) and Trinder (1951). Chloride and bicarbonate were determined using modified Skeggs and Hochestresser (1964) method and Forrester *et al* (1976) respectively.

Hematological analysis

Hemoglobin (Hb) level and erythrocyte sedimentation rate (ESR) were estimated by the methods of Alexander and Griffiths (1993). Packed cell volume (PCV) level was determined by the method of Jones (1961). White blood cell (WBC total) counts and the differentials were determined by the method of Hoffbrand and Pettit (2000).

Statistical analysis

The statistical analysis was conducted using the Student t-test as described by Steel and Torris (1960).

RESULTS AND DISCUSSION

Polluted water may contain several disease-causing pathogens (Balarajan *et al.*, 1991). When these pathogens are in the body, immune responses are triggered to neutralize and combat their disease causing effect (Balarajan *et al.*, 1991). Blood, the fluid tissue of the body houses components that play vital roles in neutralizing these pathogens and their toxins. Some of these blood components are embedded in haematological parameters and are associated with health indices (Murray, 2000). They are of diagnostic importance especially in routine clinical evaluation of the state of health (Okeke *et al.*, 2006). The haemoglobin levels (Table 1) increased significantly ($p<0.05$) in test rats against those of the control on exposure to Nworie River water. The water from the River may have contained organic pollutants that stimulated the production of haematopoietin, a glycoprotein hormone that controls erythropoiesis (Akubugwo and Duru, 2011). The significant increase ($p<0.05$) observed in PCV in test rats against those of the control rats is normal with increase in haemoglobin (Hb). Changes in haematological system have higher predictive value for human toxicity when the data are translated from animal studies (Olson *et al.*, 2000). When an antigen is introduced into an organism, antibodies are produced in response to the antigen (Akubugwu and Duru, 2011; Okeke *et al.*, 2006). The significant increase ($p<0.05$) observed in WBC counts of the test rats on exposure to Nworie River water, when compared to those of the control showed normal reaction of the test rats to foreign substances, which altered their normal physiological processes (Krishan, and Veena, 1980). The increased leucocytosis indicated a stimulation of immune system that protects the rats against infection. It may be directly proportional to the severity of the causative stress condition (Celik and Suzek, 2008). The differential count revealed significant increase ($p<0.05$) in lymphocytes count against the control. The observed increase in lymphocytes may be as a result of polluted nature of Nworie River since lymphocytes are sources of serum immunoglobulins and cellular immune

TABLE 1 Changes in some haematological parameters induced by Nworie River.

Parameter	After one month consumption						After two months consumption					
	Hb	PCV (%)	WBC	L (%)	N (%)	ESR	Hb	PCV (%)	WBC	L (%)	N (%)	ESR
Control	19.58 ± 0.08*	40.22 ± 1.06*	4205 ± 10.34*	65.12 ± 1.64*	4.30 ± 0.88	5.07 ± 0.94	20.01 ± 0.19*	44.02 ± 2.81*	4859 ± 8.22*	65.30 ± 1.60*	4.98 ± 1.33*	5.11 ± 0.12*
Test	21.92 ± 1.11*	46.03 ± 2.92*	5505 ± 4.58*	68.34 ± 0.39*	5.10 ± 1.28	6.01 ± 1.32	23.94 ± 0.36*	49.88 ± 1.01*	5720 ± 3.25*	72.13 ± 4.38*	6.94 ± 0.46*	8.09 ± 0.96*

Results are mean and standard deviation of triplicate determinations
Values asterisked (*) are statistically significant at 5% significant level
Units: Hb (mg/dl), WBC (cell/mm^2), ESR (mm/hr)

TABLE 2 Changes in liver enzymes induced by Nworie River water.

Parameter	After one month consumption					After two months consumption				
	ALT (U/L)	AST (U/L)	ALP (U/L)	TB (mg/dl)	CB (mg/dl)	ALT (U/L)	AST (U/L)	ALP (U/L)	TB (mg/dl)	CB (mg/dl)
Control	35.36 ± 0.41*	9.04 ± 0.29*	17.08 ± 1.28	1.04 ± 0.42	0.64 ± 0.03	36.09 ± 1.98*	9.84 ± 0.12*	17.13 ± 0.60*	1.54 ± 0.03	0.84 ± 0.03
Test	38.14 ± 1.09*	13.94 ± 0.87*	19.54 ± 1.66	1.12 ± 0.52	0.62 ± 0.07	40.95 ± 0.12*	13.98 ± 1.00*	20.94 ± 2.17*	1.58 ± 0.35	0.26 ± 0.01

Results are mean and standard deviation of triplicate determinations
Values asterisked (*) are statistically significant at 5% significant level.

TABLE 3 Changes induced in the kidney function by Nworie River water.

Parameter	After one month consumption						After two months consumption					
	Creatinine	Urea	K^+	Na^+	Cl^-	HCO_3^-	Creatinine	Urea	K^+	Na^+	Cl^-	HCO_3^-
Control	0.94 ± 0.12	17.39 ± 1.40*	2.01 ± 0.94*	140.33 ± 2.08	95.64 ± 1.05*	12.18 ± 0.01*	1.06 ± 0.04	18.96 ± 1.44*	2.09 ± 0.32*	141.54 ± 2.31	96.88 ± 0.12*	12.43 ± 0.05*
Test	1.09 ± 0.34	21.36 ± 0.48*	4.84 ± 0.13*	141.04 ± 1.06	99.41 ± 0.93*	19.06 ± 0.18*	1.20 ± 0.13	21.96 ± 1.44*	4.96 ± 0.59*	141.94 ± 2.31	106.41 ± 2.03*	19.87 ± 0.09*

Results are means and standard deviation of triplicate determinations
Values asterisked (*) are statistically significant at 5% significant level
Units: Creatinine (mg/dL), Urea (mg/dl), K^+/ Na^+/ Cl^- (mEq/L), HCO_3^- (mmol/L)

response and attack foreign substances directly or indirectly (Ukaga *et al.*, 2005). Neutrophils and ESR were insignificantly affected (p>0.05) in test rats when compared to those of control after a month's exposure to the River water. Both parameters were significantly (P<0.05) increased in test rats against the control rats after two months exposure. The increase in neutrophils could be that Nworie river water stimulated neutrophil production while the increase in most of the haematology parameters investigated in this study could be the cause of the reduced ESR observed after long term exposure rats.

Domestic wastes, heavy chemicals from industries, run-off from agricultural farmland, etc. are components of a polluted water body. These pollutants cause problems to health and can lead to water-borne diseases hence diseases such as skin irritation, eye irritation, ear aches, respiratory problems, diarrhoea, typhoid, cholera, dysentery (bacillary or amoebic), poliomyelitis, etc, may be inherent on exposure to such water. A large number of naturally-existing chemicals in the land or those that are added due to human activity dissolve in water leading to pollution and various diseases (Prieto *et al.*, 2001). Some of the heavy chemicals that pollute water impair the liver on consumption by damaging it through diseases. Infective hepatitis, liver abscission, cancer, etc., are possible liver infections contracted through consumption of polluted water (Favero, 1985). Liver infection leads to liver tissue damage (Enermor *et al.*, 2005), and liver tissue damage is usually associated with the release of enzymes specific to the tissue (Aliyu *et al.*, 2006). Friday (2004) noted that cell-derived enzymes have high activity in cells and spill in plasma when the cell is damaged or the enzymes produced in excess. ALT and AST (Table 2) increased significantly (p<0.05) in test rats against those of the control on exposure to water from Nworie River. Nworie River could be the cause of these enzymes linkage. As a polluted water body, it may contain substances that are injurious to the liver cells. Enermor *et al.* (2005) noted that small increase in ALT and AST might be due to the wide range of liver disease. It follows that Nworie River water may have induced diseases which ruptured the tissues peculiar to these enzymes hence the significant increase observed in test rats in the present study. Both ALT and AST are excellent markers of liver damage caused by exposure to toxic substances (Ranjna, 1999), although AST is a less specific indicator of liver function (Akubugwo and Duru, 2011; Aliyu *et al.*, 2006; Moss and Henderson, 1996). In a related study, Amadi *et al.*, (2006) had similar observation on liver function of rats given water from Nworie River. ALP occurs in the liver next to bile ducts and in the bone, and leaks into the bloodstream in a way similar to that of transaminases (Akubugwo and Duru, 2011; Friday, 2004; Mathew, 2001). ALP increased significantly (p<0.05) in test rats against those of the control in the present study. Total and conjugated bilirubin was not significantly affected (p>0.05) in test rats when compared to those of the control on exposure to Nworie River water. This may imply that consumption of the river water could not be linked to diseases such as jaundice (Brown, 2007).

In contrast to tests for microbial loads and some physicochemical parameters, which can be done to determine water quality, toxic cocktails of pesticides, herbicides, some heavy metals, etc, are not easily determined (NEPA, 1996). These

substances tend to accumulate in the body. Filtration, regulation and excretion of these toxic materials are done in the kidney. Hence the organ is responsible for the regulation of total internal environment (Homeostatic function) of the body (Hyman, 2004). Kidney function is affected on exposure to water polluted with bio-accumulating substances. Accumulation of such substances in the kidney results in disease, which may finally end in kidney failure if not properly handled (Markandya, 2004). Due to possible kidney diseases inherent from consumption of polluted water, kidney function parameters are used as indices to mark its healthy state. Creatinine is a waste product formed by creatine metabolism. It is the major catabolic products of the muscle and is excreted in the kidney. The serum creatinine levels are used as an indicator of renal failure (Friday, 2004; Nsirim, 1999). Rats placed on Nworie river water (Table 3) had no significant difference in creatinine (p>0.05) when compared to those of the control. Urea is the main end product of protein catabolism. Urea varies directly with protein intake and inversely with the rate of excretion (Ranjna, 1999). Renal diseases that diminish the glomerular filtration lead to urea retention and decrease in urea is seen in severe liver diseases (Wurochekke *et al.*, 2008). In this study, rats placed on water from Nworie River showed significant increase (p<0.05) in urea when compared to those of the control. Nduka (1999) noted that high blood urea is associated with increased tissue protein catabolism, excess breakdown of blood protein and diminished excretion of urea. The significant increase (p<0.05) observed in potassium ion, chloride, and bicarbonate in test rats against those of the control could be that Nworie river water had a stimulating effect on aldosterone hormone in the system of test rats. The studied river water had no effect on potassium ions. The insignificant increase (p>0.05) in creatinine in test rats observed in the present study could be an indication that the hepatocellular damage induced by water from Nworie river may not be severe in test rats.

CONCLUSION

The present study has shown that long-term consumption of water from Nworie River induces haematological, renal function, hepatic function changes in rats. Since data obtained with animal studies have higher predictive value for human toxicity when translated, it therefore means that long-term consumption of water from Nworie River could be toxic to humans. The findings of the present study would suggest that the local public health authority should conduct a descriptive survey to find the estimated number of people dependent on this water source. The public water supply authority within the Owerri municipality should improve in service provision to people within the municipality.

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