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THE EFFECTS OF CHLORINATED DRINKING WATER ON PIG'S SPLEEN DNA TREATED WITH ZINC SULPHATE AND GLUTATHIONE

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ABSTRACT: *Background and Aim: Disinfection of surface waters is often done to destroy pathogenic organisms present in water bodies in order to render water safe for consumption, but chlorination of drinking waters has been debated as being toxic to experimental animals including man. The aim of this work was to investigate using biochemical techniques whether disinfection of drinking water by chlorination is harmful or not. Materials and Methods: The materials used were water, calcium hypochlorite, Pig's spleen, zinc sulphate and reduced glutathione. The water sample was obtained from river Jama'are in Bauchi state, and its quality assessed by estimation of temperature, pH, total dissolved solids, total hardness,* Cl^-, SO_3^{2-}, Na^+, K^+, Ca^{2+}, *and* Mg^{2+} *contents. The water sample was chlorinated using calcium hypochlorite (65-70%* Cl_2*). DNA was isolated from Pig's spleen and used as a model to test the direct effect of the chlorinated water sample on animal health. The effect was monitored spectrophotometrically. The effect of the chlorinated water sample on the biomolecule was also studied in the presence of zinc sulphate and glutathione (GSH). This was compared with that of a control experiment. Results: The results revealed that the chlorinated water sample altered the native structure of the isolated DNA. But in the presence of 0.1mM zinc sulphate and varied concentrations of GSH (0.1mM, 0.2mM and 0.3mM respectively) the chlorinated sample was found to have no noticeable effect on the isolated biomolecule; a pointer that Zn and GSH may have conjugated with chlorinated water products and detoxified them. Conclusion: It is suggested that drinking of chlorinated water is not harmful to health.*

KEYWORDS: Water, Chlorination, DNA, Zinc, Glutathione, Pig, Health

INTRODUCTION

Water is one of the essential amenities needed for daily survival. Its main sources include; well, river, rain, taps just to mention but a few. Most of these water bodies are polluted as a result of both natural processes and human activities. Thus, to make water safe for human consumption and use, the unwanted contents in surface waters should be removed. Clinton (2003) highlighted the process of purification of surface waters to include flocculation to precipitate particulate materials, filtration to remove the particulate matter, and disinfection to eradicate pathogens. Both the flocculation and filtration processes are mechanical while disinfection involves the use of chemical and biochemical principles for effectiveness.

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There are several disinfectants that have been applied in water treatment as reported previously (Stringer *et al*., 1995; and Godwin, 2003). These disinfectants include the use of chlorine (chlorination), ozone (ozonation), iodine, bromine and UV light. Of these, chlorination is the most common technique used because of its germicidal and bactericidal effectiveness (Smith and Sparkman, 2001). The use of chlorine in water treatment is affected by several factors such as; dissolved organic matter and readily oxidizeable compounds in the water which neutralize disinfectants. Several studies have shown that suspended matter could exhibit a shielding effect on bacteria against the disinfectant (Okuofu and Dada, 1990; Ikka, 1997; and Randy, 2005). However, test conditions like pH, temperature, and chemical characteristics of the water could also be considered in determining the disinfectant efficiency, (Randy, 2005). The overall objective of disinfection is to ensure that the quality criteria of drinking water, is always met based on the World Health Organization standard. But chlorination has been reported to result in the formation of chlorinated organic compounds including 3-chloro-(dichloromethyl)-5-hydroxy-2[H]-furanone (MX), trihalomethanes (THMs), halogenated acetonitrile (HAN), dichloroacetonitrile (DCAN), chloroform, carbon tetrachloride, chloral (trichloroacetaldehyde), chlorobenzene, dichloroethane, dichlorophenol, hexachloroethane, methylchloride, methylene chloride, polychlorinated biphenyls and venylchloride which have been documented to be toxic to experimental animals and man (Hemming *et al.,* 1999; William *et al.,* 2000; and Becham *et al.,* 2002). Besides, Esemikose and Olayemi (2011) are of the views that though the use of chemical disinfectants like chlorine results in the formation of some chemical-by- products some of which are hazardous to health, the risk posed to health are extremely small in comparison with those associated with inadequate disinfection. They argued that disinfection should not be compromised by attempts to control such by- products.
The aim of the present study therefore was to investigate whether disinfection of drinking water by chlorination is harmful to health or not.

MATERIALS AND METHODS

Water sample was obtained from river Jama'are in Jama'are local government area of Bauchi state, Nigeria. The river serves as a source of drinking water to some people in the area. The water sample was collected using WHO (2006) guidelines, and was done in the month of July 2012 in the morning hour (9.30am). The temperature of the water was recorded immediately and later transferred to the laboratory for analysis. Physicochemical parameters test on the water sample was conducted using the procedures of Randy (2005). Calcium hypochlorite (65-70% Cl_2) was obtained from Bauchi State Water Board, Bauchi, Nigeria. The sample was chlorinated using the calcium hypochlorite following the steps of (Ubom *et al*., 1993 and Shariff, 2003).

Deoxyribonucleic acid (DNA) was isolated from Pig's spleen using the methods of Pollister and Joseph (1992) as modified by Ebe and Ubom (2011), and used to test the direct effect of chlorinated water sample *in vitro*. Prior to this, the isolated biomolecule was initially characterized using temperature profiling (denaturing and renaturing processes) following the procedures of Stein (1994). The effect was monitored spectrophotometrically by adopting the

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method of Dewam and John (1994). The effect of the chlorinated water sample on the biomolecule was also studied in the presence of zinc sulphate and glutathione (GSH). The experiment was arranged as follows:

(i) A portion (10mg) of the isolated DNA was dissolved in 100cm^3 of saline buffer pH 7.4 to serve as control.

(ii) Similar DNA solution as in (i) was again prepared and 0.1mM of zinc sulphate was added. This experiment was repeated three times and placed in three separate beakers. Different concentrations of GSH (0.1mM, 0.2mM and 0.3mM) were dissolved respectively in the derived DNA-zinc mixtures to serve as control.

(iii) Test samples in (ii) were again prepared, and 100cm^3 of the chlorinated water sample was added to each beaker.

All the test samples were left in the refrigerator overnight and thereafter, absorbance was read at 220 - 440nm against a blank of buffered saline pH 7.4. All reagents used for the analysis were of analytical grades.

RESULTS

The results of absorption of the isolated Pig's spleen DNA at 220 - 440nm are presented in Figure 1. Here, the isolated biomolecule shows absorption maxima at 220 and 260nm and minima at 300, 410 and 440nm.

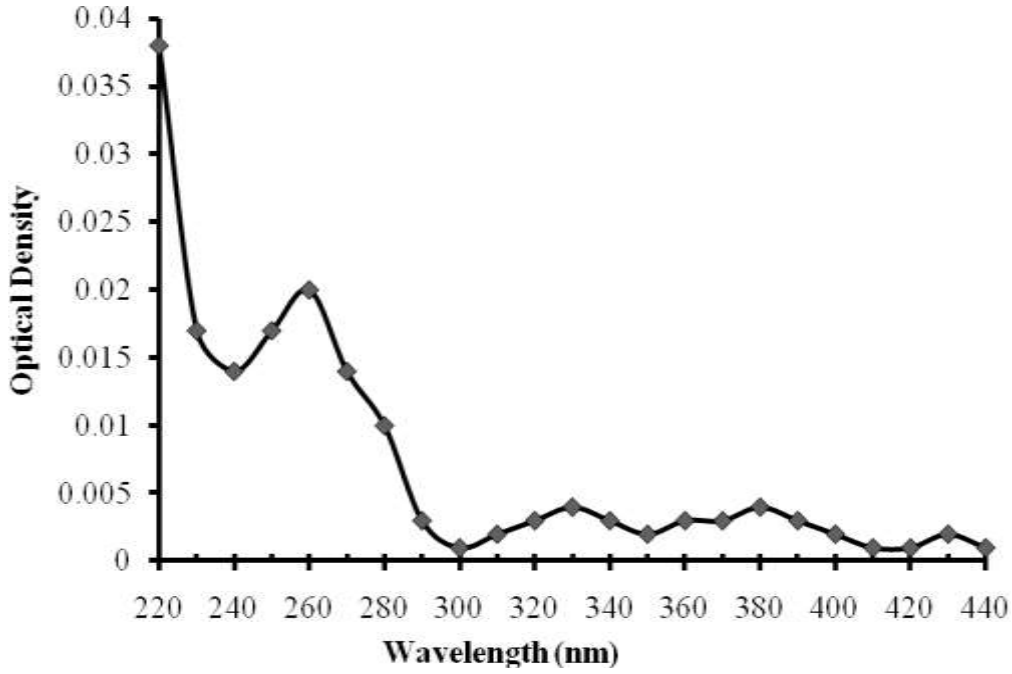

Figure 1: Absorption spectrum of isolated DNA from Pig's spleen

The result of the effect of temperature on the extinction of the isolated DNA is shown in figure 2. The result reveals that heat had effect on the isolated Pig's spleen DNA. The result also

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shows that cooling the hot DNA solution slowly had no significant effect on the isolated biomolecule, but rapid cooling had a significant effect on the isolated DNA.

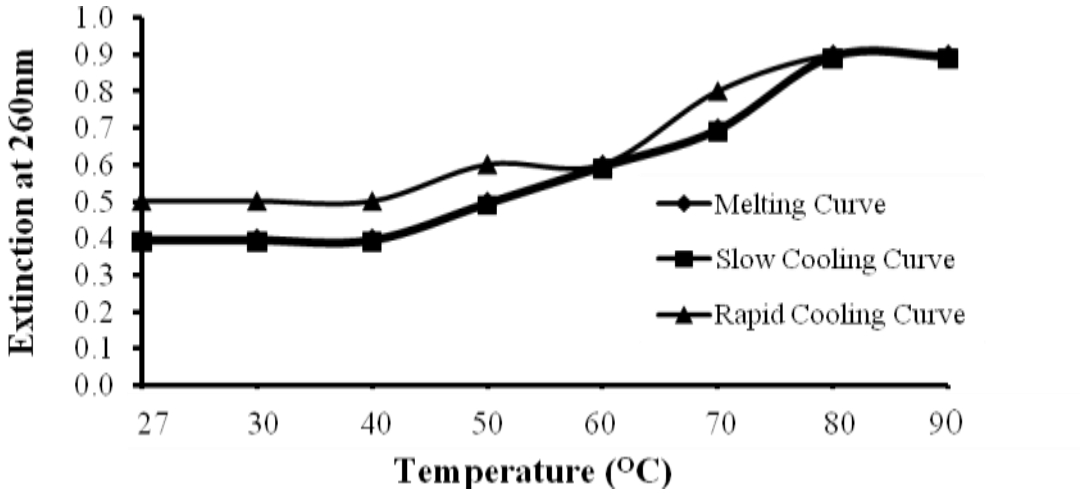

Figure 2: The effect of temperature on the extinction of DNA isolated from Pig's spleen

The physicochemical parameter of the water sample under which the test was conducted is shown in table 1.

Table 1: Mean value of physicochemical parameter of the Water sample

Parameter	Mean value	WHO (2004) Standard Values for Drinking Water
Temperature (°C)	28.0	12 - 25
pH	8.6	6.5 - 8.5
Total Dissolved Solid (mg/l)	48.5	500 - 1000
Total hardness (mg/l)	34.1	500
Cl^- (mg/l)	0.3	250
SO_3^{2-} (mg/l)	2.5	250
Na^+ (mg/l)	12	200
K^+ (mg/l)	6.4	20
Ca^{2+} (mg/l)	14	100
Mg^{2+} (mg/l)	11.3	50

Results are means of duplicate determinations.

Figure 3 displays absorption of the chlorinated water sample at wavelength 220 – 440nm. The result shows absorption maxima at 230nm and minima at 430 - 440nm.

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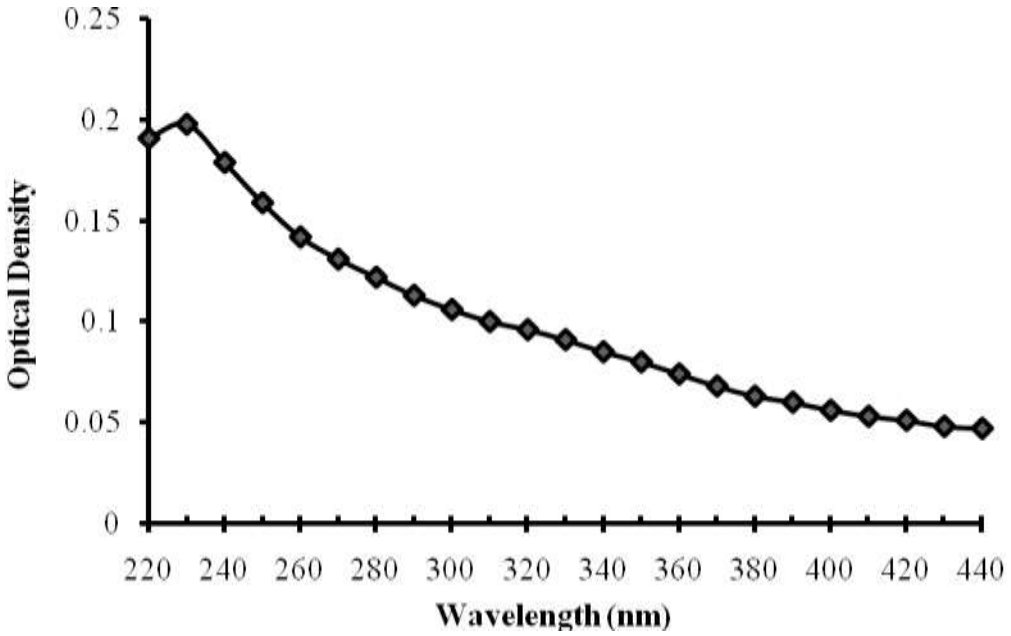

Figure 3: Absorption spectrum of chlorinated Water sample

Similarly, the result of absorption of the isolated DNA in the presence of chlorinated water sample at wavelength 220 – 440nm is presented in figure 4. Here, absorption maxima are at 220nm and 260nm, and minima at 440nm.

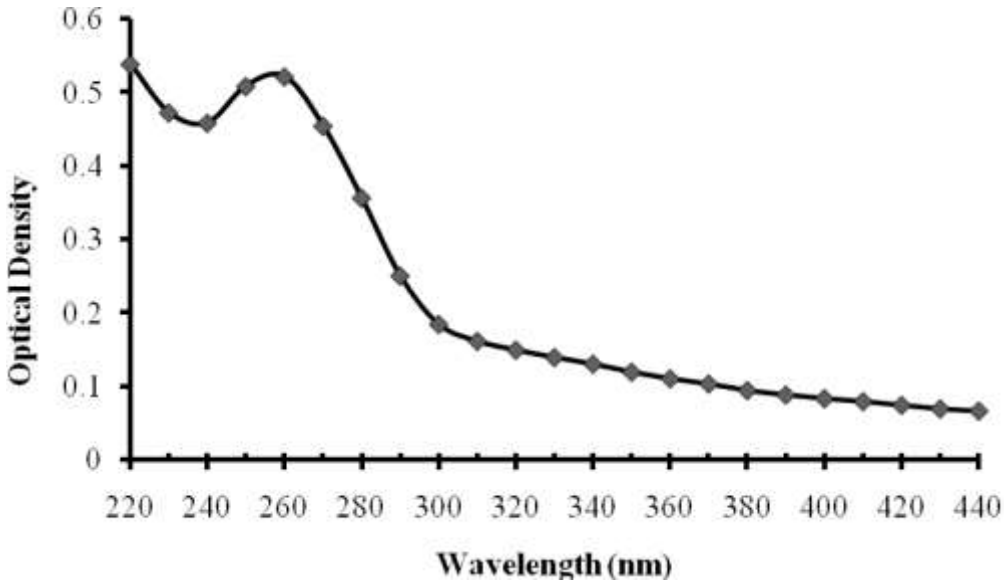

Figure 4: Absorption spectrum of isolated DNA plus chlorinated Water sample

The results of absorption of the isolated DNA in the presence of zinc sulphate and GSH (control) at wavelength 220 – 440nm are displayed in figure 5. Here, maximum absorption is at 220nm and minima between 300 – 440nm.

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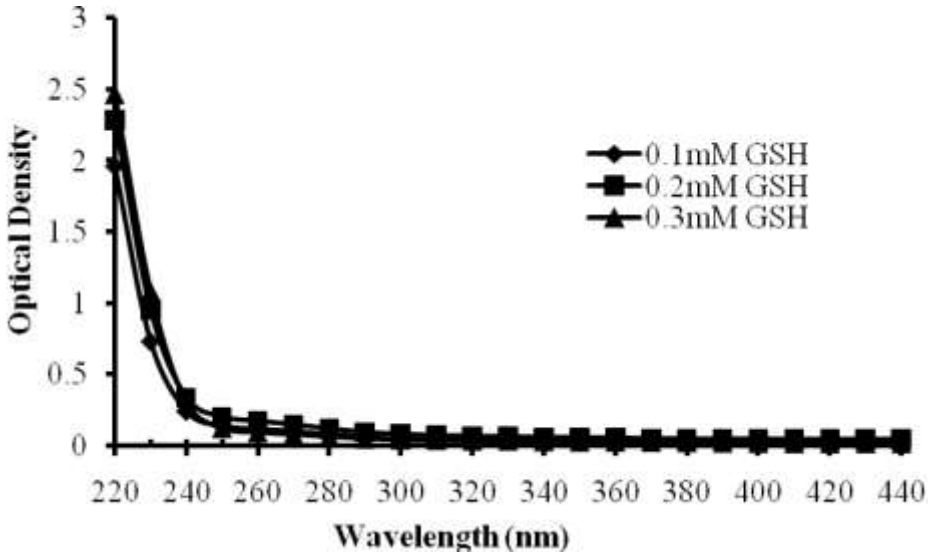

Figure 5: Absorption spectra of isolated DNA plus Zinc and GSH (control)

Also, the results of absorption of the isolated DNA in the presence of zinc sulphate, GSH and chlorinated water sample at wavelength 220 – 440nm are shown in figure 6. Here, maximum absorption is at 220nm and minima between 320 – 440nm.

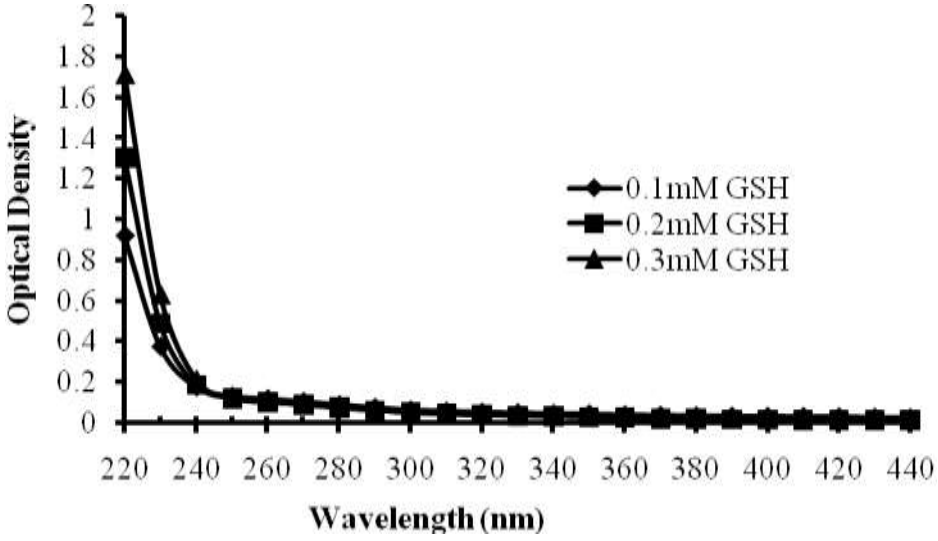

Figure 6: Absorption spectra of isolated DNA plus Zinc, GSH and chlorinated Water sample

Discussion

The physico-chemical characterization of the water sample is given in Table 1. The mean temperature value was found to be 28ºC. This value crossed the prescribed range of WHO

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(2004) for drinking water. The high temperature could be attributed to the climatic factors prevailing at the time of sampling. The mean pH value of the water sample was 8.6. The water was showing slightly alkaline nature and not within the safe limit. Total dissolved solids (TDS) was found to have a mean value of 48.5 mg/l. This value was not within the prescribed limit. High concentration of salts of sodium, calcium and magnesium is generally responsible for high concentrations of TDS. The total hardness caused by sulphates of calcium and magnesium and chloride recorded a mean value of 34.1 mg/l. A number of diseases correlated with water hardness include nervous system defects, various types of cancers and prenatal mortality (Ikka, 1997). Chloride occurs in all natural waters in widely varying concentration. Chloride normally increases as the mineral contents increases (Clinton, 2003). In this present study, the mean value of chloride concentration of the water sample 0.3mg/l was found to be below the prescribed limit. Similarly, the mean values of sulphate, sodium, potassium, calcium and magnesium ion concentrations of the water sample investigated were 2.5, 12, 6.4, 14 and 11.3mg/l respectively. These values were not within the prescribed limits.

The results also show that the isolated Pig's spleen DNA absorbs light strongly in the region of the spectrum (220 - 440nm) due to the conjugated double bonds systems of the constituent purines and pyrimidines. It shows characteristics maxima at 250nm and 260nm. This is in conformity with known and established properties of the biomolecule (Sharif, 2003). Besides, when a solution of the isolated DNA was slowly heated, there was a little change in absorbance until the melting temperature (Tm) of 75^{o}C was reached; at this stage, absorbance increased rapidly to a higher value of 0.900 which was not significantly changed by further heating. At the melting temperature of 75^{o}C, the hydrogen bonds between base pairs were broken and the two strands were separated (Stein, 1994).

On cooling the hot DNA solution slowly, the two strands recombined (i.e. the 'cooling curve' overlapped on the 'melting curve'). But on cooling the hot DNA solution rapidly, the rapid cooling curve did not overlap on the melting curve, and the melting temperature also was different. This suggests that the denaturation and renaturation processes did not coincide (figure 2). This initial analysis became necessary with the isolated biomolecule to ascertain whether it conforms to known and established properties of native DNA before using it for the study.

The results from this work showed that the chlorinated drinking water sample studied caused damage to isolated Pig's spleen DNA *in vitro* in the absence of Zn and GSH, by altering its native structure (figures 1, 3 and 4). This finding agrees with the studies conducted by Hemming *et al.,* (1999); and William *et al.,* (2000), in which chlorination of surface waters was reported to result in the formation of chlorinated organic compounds such as halogenated acetonitrile (HAN), carbon tetrachloride, chloral (trichloroacetaldehyde), chlorobenzene, hexachloroethane, methylene chloride and polychlorinated biphenyls which were found to be toxic to experimental animals. Further findings from the present work revealed that addition of Zn and GSH to the isolated DNA resulted in a conformational modification which was found to have preserved the native DNA from damage upon interaction with the chlorinated water sample (figures 5 and 6). This could suggest that Zn and GSH may have conjugated with

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chlorinated water products and detoxified them. These findings could be used to mimic what takes place in biological systems. In mammals for instance, the liver is known to contain GSH (5-10mM in rat liver, and about 4mM in humans) Gutman (2002), and zinc is also known as a component of many different enzymes that function in almost every aspect of cellular metabolism and at the same time, needed for proper functioning of the immune system (Brian *et al.*, 2003). After drinking chlorinated water, the chlorination products get to the liver where they could be conjugated with GSH and thereafter get detoxified and removed via urine (water soluble) or the bile (fat soluble).

Zn and GSH have been found by this study to be very potent in conjugating pollutants resulting from water chlorination. The present work however shows evidence that Zn and GSH, both abundant in cells, could provide a system which is important in the detoxification of chlorination products having the potential for cytotoxic or genotoxic damage. This finding is a pointer to the fact that the unnecessary anxiety surrounding drinking of chlorinated water is uncalled for, since the antioxidants (Zn and GSH) identified by this study are suggested to be capable of conjugating the products of chlorination of drinking waters *in vivo* and prevent them from causing harm to cellular DNA. The present finding is also a confirmation to the assertion made by Esemikose and Olayemi (2011) that disinfection of drinking water by chlorination should not be compromised by attempts to control the purported harmful chemical- by-products resulting from the process. The present study however reveals that the level of cellular glutathione should be kept high to ensure continuous detoxification and removal of pollutants from the body. Bad health and poor nutrition can weaken the body immune system in fighting pollutants from drinking water, air or food. But good nutrition may supply the needed antioxidants required by the body cells for healthy living. The 'magic' antioxidants discovered by this study can be obtained locally from food materials.

CONCLUSION

It is established from this study that though the chlorinated water sample caused damage to the isolated Pig's spleen DNA *in vitro*, in the absence of Zn and GSH, drinking chlorinated water may not cause harm to cellular DNA (*in vivo*) since Zn and GSH are readily available in cells to conjugate and destroy pollutants resulting from water chlorination. This work therefore suggests that disinfection of drinking water by chlorination is not harmful to health as long as an individual remains in good nutritional state. It further recommends that the amino acids needed for synthesis of GSH (glutamic acid, glycine and cysteine) in the cells (humans) should be supplemented regularly. This, the study suggests can be done by consuming important dietary sources of this antioxidant such as fresh fruits and vegetables, fish and meat. In addition, since antioxidants do not function alone, it is equally advisable based on this study to be consuming zinc-rich foods such as chicken, nuts and legumes regularly along with normal meals.

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