



***Moringa oleifera* seed powder prevents untreated Asa River water-induced oxidative stress in rats**

**¹Oloyede, H. O. B., ¹Bamgbade, A. A. , *¹Salawu, M. O., ¹Nafiu, M. O.,
²Ajiboye, T. O. ³Adegoke, M. B.**

¹Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.

²Department of Biological Sciences, Al-Hikmah University, Ilorin, Nigeria.

³Department of Applied Sciences, Osun State Polytechnic, Iree, Nigeria

Abstract: There is need to find safe and cheap means of treating water for drinking at household level due to the dangers in use of chemicals for treating water. *Moringa oleifera* (MO) seed powder was used as a coagulant to treat Asa River Water (ARW). The effect on the liver and kidney of albino rats (*Rattus norvegicus*) induced by the untreated ARW and the treated ARW were studied. Twenty rats were randomly grouped into four. Group A was exposed to tap water while groups B to D were exposed to MO seed powder-treated ARW, ARW and MO-seed powder-treated tap water respectively. At the end of twenty-eight (28) days exposure, the alkaline phosphatase (ALP), acid phosphatase (ACP), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GSH-Red), glucose-6-phosphate dehydrogenase (G-6-PD) and reduced glutathione (GSH) activities as well as malondialdehyde (MDA) concentrations of the liver and kidneys of the rats were determined. Histological examinations of the tissues were also done. There was significant ($p < 0.05$) decrease in specific activities of the tissue ALP, ACP, SOD, CAT, GPx, GSH-Red and G-6-PD, and a significant ($p > 0.05$) increase in MDA concentrations in group A rats compared to the controls. The rats in MO seed powder-treated ARW group have specific activities of ALP, ACP, SOD, CAT, GPx, GSH-Red and G-6-PD with no significant difference from the normal control. The liver and kidney GSH, and MDA concentrations exhibited no significant difference from those of normal control. There was tissue deterioration in the liver and kidney of rats exposed to ARW while those of normal control rats and MO-treated ARW rats appeared normal.

KEYWORDS: Antioxidant enzymes, Asa River, *Moringa oleifera*, Oxidative stress

1.0 Introduction

It has been estimated that 85% of the world population live in the arid zones of the planet, while 783 million people do not have access to clean water and almost 2.5 billion do not have access to adequate sanitation. Annually, 6 to 8 million people die from the consequences of disasters and water-related diseases (www.unwater.org, 2013). In the poor countries in Africa, Asia, and Latin America, approximately 1.4 billion rural and peri-urban people live in extreme poverty (<\$1.25 U.S./day). Majority of this population relies almost exclusively upon traditional sources of

highly turbid and untreated pathogenic (viral and bacterial) surface water for their domestic water needs (Chen and Ravallion, 2008).

A relatively recent survey by Majure *et al.* (2011) showed that 65 million Nigerians which include 1.8 million Kwara State residents constituting 2.8% of Nigeria's population had no access to safe water. In Ilorin, the capital of Kwara State the Asa River water is used for domestic purposes by the majority of the inhabitants. The river receives effluents from the industries, agricultural and domestic activities in the area. The report by Alaya *et al.*, 2010 indicates heavy metal enrichment of the Asa River water with cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn) and these metals

***Corresponding Author**

Tel.: +234 (0) 8056168556

E-mail: musasalawu@yahoo.com

have been found to exceed the maximum permissible concentration for drinking water. Dependence on such water for domestic purposes, particularly for drinking by man and other living organisms may induce plural changes including oxidative stress (Li and Brouna, 2006). The oxidative stress so induced may activate the endogenous production of reactive oxygen species (ROS) and consequently enhance ROS-mediated oxidative damage.

Several chemical coagulants including inorganic (like Alum), synthetic organic polymer and naturally occurring coagulants have been used in conventional water treatment processes for portable water production. Generally, alum (Aluminum sulphate) and its synthetic polymeric derivatives are widely used in water treatment (Majure *et al.*, 2011). However, there is fear that alum may have strong carcinogenic properties. On the other hand, there is evidence that the use of extracts from plant species possessing both coagulating and antimicrobial properties is safe for human health (Olayemi and Alabi, 1994). A typical example of this is *Moringa oleifera* seed powder. When mixed with water, the crushed seed powder or residue presscake yields water-soluble organic polymers acting as flocculating agents, binding suspended particles in a colloidal suspension forming large sedimenting particles (flocs) (Dorea, 2006). However, the use of extracts from plants possessing both coagulating and antimicrobial properties has been reported to be safe for human health. The challenge for us is to recognize, promote, and adopt proven indigenous methods that remove the most turbidity at the lowest cost, producing water of low risk for marginalized households (Marobhe, 2008).

Asa River water in Ilorin area of Kwara State was chosen for the present study because the inhabitants of the area depend on it for domestic purposes not minding the extent of the pollution of the water. Although, documentation has been made of the induced oxidative stress associated with consumption of the Asa River water but no report has been on the possible preventive activity of *Moringa oleifera* seed powder on the Asa River-water-induced oxidative stress.

The overall Objective of this study is to assess the preventive activity of *Moringa*

oleifera seed powder on Asa River water-induced oxidative stress.

2.0 Materials and Methods

2.1 Materials

2.1.1 Chemicals

5,5'-Dithio-bis(2-nitrobenzoic acid), p-Nitrophenyl phosphate, disodium salt, hexahydrate and guanidine hydrochloride, Ultra Pure were products of Research Organics, Cleveland, Ohio 44125. All other reagents used were supplied by Sigma-Aldrich Inc., St. Louis, USA.

2.1.2 Assay Kits

Glutathione peroxidase, glutathione reductase and glucose 6-phosphate dehydrogenase assay kits were products of Randox laboratories Co-Antrim, United Kingdom.

2.1.3 Plant Material and Authentication

Moringa oleifera seeds were obtained at Oke-Odo area of Ilorin, Kwara State, Nigeria. The plant sample was authenticated and deposited in the Herbarium of the Department of Plant Biology, University of Ilorin, Kwara State, Nigeria. Voucher Number I.U.H.V Number 13 was issued in respect of the sample.

2.1.4 Animals

Twenty apparently healthy male albino rats (*Rattus norvegicus*) weighing averagely 135 ± 2.14 g were obtained from Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Kwara State, Nigeria. They were kept in clean plastic cages contained in well-ventilated house conditions with free access to feeds (Bendel Feeds Ltd., Taiwo Road, Ilorin, Kwara State, Nigeria) but exposed to different forms of water.

2.2 Methods

2.2.1 Physicochemical properties of Asa River Water

The Asa River water was analyzed for their physicochemical properties in accordance with standard methods (APHA, 1985). The Nephroturbidity Test was carried out spectrophotometrically using Digital spectrophotometer while the metals were assayed using the Atomic Absorption Spectrophotometer (ASS).

2.2.2. Animal Grouping and Management

Apparently twenty healthy male rats were completely randomized into four groups (A-D) of 5 animals each and each group was placed on different water samples as follows:-

Group A: Animals exposed to tap water (Normal control).

Group B: Animals exposed to *Moringa oleifera* seed powder-treated Asa River water.

Group C: Animals exposed to Asa River water.

Group D: Animals exposed to *Moringa oleifera* seed powder-treated tap water.

2.2.3 Preparation of Serum and Tissue Homogenates

The rats were sacrificed 24 hours after the last day of treatment by placing them in jar containing wool soaked in diethyl-ether. After they were unconscious their jugular veins were sharply cut with clean sterile scalpel blade to collect the blood. The blood sample collected were allowed to clot and then centrifuged at 300 g for 5 minutes for serum preparation using centrifuge. The clear supernatant was used for determination of the estimation of liver and kidney enzymes activities.

Liver and kidneys removed from the rats were cleaned and immersed in ice-cold 0.25M sucrose solution to maintain the integrity of the organ. It was blotted with tissue paper, cut thinly with sterile scalpel blade and then homogenized in ice-cold 0.25M sucrose solution. The homogenates were then frozen overnight to ensure the maximum release of the enzymes located in the cell before being used for the enzyme assay.

2.2.4 Determination of Biochemical Parameters

Alkaline phosphatase activity was assayed following the method described by Bernet *et al.*, (1974). Acid phosphatase assay was carried out following the method reported by Bergmeyer *et al.* (1974). Superoxide dismutase (SOD) and Catalase (CAT) were assayed as reported by Misra and Fridovich (1975) and Sinha (1972) respectively. Glutathione Peroxidase (GPX), Glutathione reductase (GSH-Red) (Paglia and Valentine, 1967) and Glucose-6-phosphate dehydrogenase (Glu-6-PD) were determined as described by Goldberg *et al.*, (1961). Reduced glutathione (GSH) and malondialdehyde (MDA) concentrations were determined according to the methods of Ellmann (1959) and Nelson (2004) respectively. The protein content in the tissues and serum of the animals were determined as described by Gornal *et al* (1949).

2.2.5 Data Analysis

Analysis of variance (ANOVA) followed by DUNCAN's Multiple range test were used to detect difference between means as well as the interactions between the variables, using SPSS 20. Differences were considered statistically significant at $p < 0.05$.

3.0 Results

The Nephroturbidity of the Asa River water was much higher (160 NTU) than the WHO limits. Other Parameters such as pH and Total Solids also indicated figures above the WHO limits (Table 1). Concentrations of the heavy metals (Pb, Ar, Cd, Cr, Co, Hg, Mn, and Fe) in the Asa River water were also observed to be higher than the WHO standards. There was no significant ($p < 0.05$) difference in the water consumption of the four groups of the experimental rats (Figure 1). Exposure of the rats to Asa River water resulted to a significant ($p > 0.05$) increase in the liver to body weight as well as kidney to body weight ratios relative to normal control (Figure 2). However, liver to body weight ratio as well as the kidney to body weight ratio of the rats exposed to *Moringa oleifera* seed powder-treated Asa River water showed no significant difference from those of normal control rats (Figure 2). Similarly, the liver to body weight as well as the kidney to

body weight ratios of the rats exposed to *Moringa oleifera* seed powder-treated tap water showed no significant difference from those of normal control rats.

There was a significant ($p < 0.05$) decrease in the specific activities of the liver and kidney ALP and ACP accompanied by a corresponding significant ($p > 0.05$) increase in the serum ALP and ACP of the rats exposed to Asa River water when compared with those of normal control (Figures 3 and 4 respectively). Even though, the specific activities of ALP and ACP of the rats exposed to *Moringa oleifera* seed powder treated Asa River water showed no significant difference from those of normal control rats, the exposure of the rats to *Moringa oleifera* seed powder treated tap water resulted to a significant ($p > 0.05$) increase in the specific activities of the liver and kidney ALP and ACP, accompanied by a significant ($p < 0.05$) decrease in the specific activities of the serum ALP and ACP (Figures 3 and 4 respectively).

The specific activities of the antioxidant markers (SOD, CAT, GSH-Px, GSH-Red, and G-6-PD) decreased significantly ($P < 0.05$) in the liver and kidney of Asa River water exposed rats when compared with those of the Normal control (Figures 5, 6, 7, 8, and 9 respectively). However, the specific activities of the antioxidant markers (SOD, CAT, GSH-Px, GSH-Red, and G-6-PD) of the rats exposed to *Moringa oleifera* seed powder-treated Asa River water showed no significant difference from those of Normal control rats, exposure of the rats to *Moringa oleifera* seed powder-treated tap water resulted in a significant ($P > 0.05$) increase in the specific activities of the antioxidant markers (SOD, CAT, GSH-Px, GSH-Red, G-6-PD and GSH) in the liver and kidney of rats (Figures 5, 6, 7, 8, 9 and 10 respectively).

Similarly, the concentrations of GSH of the rats exposed to *Moringa oleifera* seed powder-treated Asa River water showed no significant difference from those of control rats, exposure of the rats to *Moringa oleifera* seed powder-treated tap water resulted in a significant ($P > 0.05$) increase in the concentrations of the antioxidant marker (GSH) in the liver and kidney of rats (Figures 10).

Exposure of the rats to Asa River water resulted to a significant ($p > 0.05$) increase in the liver and kidney MDA concentrations relative to those of the Normal control rats (Figure 11).

Even though, the concentrations of liver and kidney MDA in rats exposed to *Moringa oleifera* seed powder-treated Asa River water showed no significant difference from those of the control rats. Exposure of the rats to *Moringa oleifera* seed powder-treated tap water resulted to significant ($p < 0.05$) decrease in liver and kidney MDA Concentrations relative to the rats exposed to tap water (Control) (Figure 11).

4.0 Discussion

Water pollution is a major global problem affecting developing countries including Nigeria (Majure *et al.*, 2011). A more serious aspect of water pollution is that caused by human activities and industrialization, leading to the release of harmful substances into the water bodies. Exposure of humans to these mixtures of hazardous substances induces oxidative stress (Dickinson and Chang, 2011). Yet, safe drinking water is a fundamental human right as much as the right to clean air (Lead, 2010). Several chemical coagulants have been used in conventional water treatment for production of potable water. The carcinogenic properties of these chemicals and their high costs especially in developing countries makes it imperative to look for cheaper alternatives that are environment-friendly and safe (Folkard and Sutherland, 2001).

The use of extracts from plants possessing both coagulating and antimicrobial properties has been reported to be safe for human health. A typical example of this is *Moringa oleifera* seed powder (Dorea, 2006). The challenge for us is to recognize, promote, and adopt proven indigenous methods that remove the most turbidity at the lowest cost, producing water of low risk for marginalized households (Marobhe, 2008).

The findings as revealed by the physicochemical characteristics of Asa River water (Table 1) lend credence to the status of the water as being unacceptable for human consumption (WHO, 2009). This is partly espoused by the level of nephroturbidity of the

Table 1: Physico-chemical Characteristics of Asa River Water

Parameters	Asa water (mg/l)	USEPA (mg/l)	WHO (mg/l) MPC
pH ⁺	8.40	6.5	NM
Colour ⁺	Pale brown	Colourless	
BOD	1.57	NS	NS
COD	1.94	NS	NS
Total Solids	2.7422	1.500	1.5
Total Dissolved Solids	1240.0	NS	NS
Nephroturbidity Unit	160	0.5-1	NGL ⁺
Copper	-----	1.30	2.0
Lead	0.019	0.015	0.010
Cadmium	0.072	0.050	0.010
Arsenite	0.140	0.010	0.010
Cobalt	0.022	---	---
Chromium	0.18	0.1	0.050
Mercury	0.056	0.002	0.002
Silver	0.004	----	-----
Zinc	0.071	5.0	5.0
Manganase	0.0848	0.050	0.040
Iron	0.0322	0.030	NGL ⁺⁺

⁺:Not in mg/l; MPC: Maximum Permissible Concentration; NS: Not Specified; NGL⁺: No Guide line but desirably less 5NTU

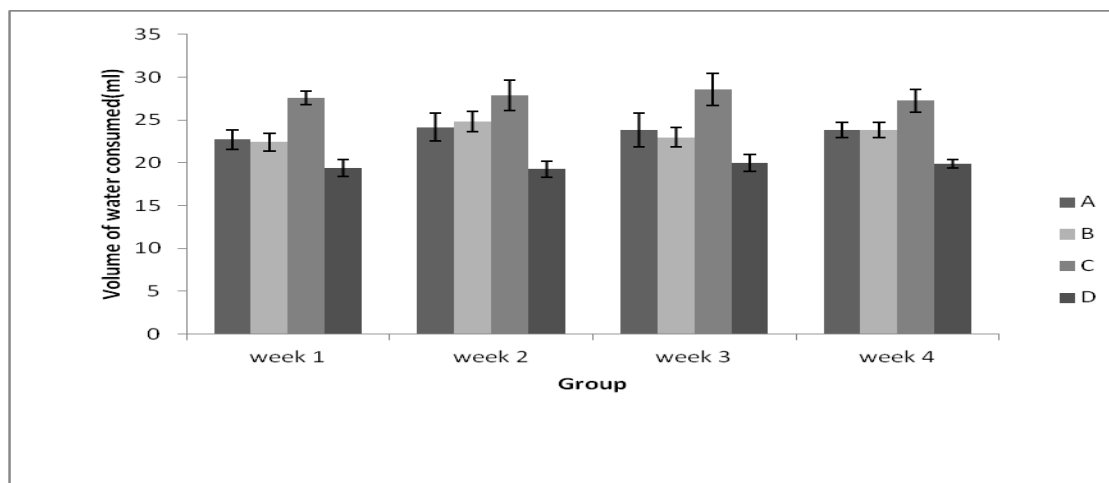


Figure 1: Weekly Water Consumption by the various groups of Albino rats

Values are Mean $\pm$ SEM; Bars with different letter have values that are significantly different ($p < 0.05$).

Key: A: Animals exposed to tap water. B: Animals exposed to *Moringa oleifera* seed powder-treated Asa River water. C: Animals exposed to Asa River water. D: Animals exposed to *Moringa oleifera* seed powder treated tap water

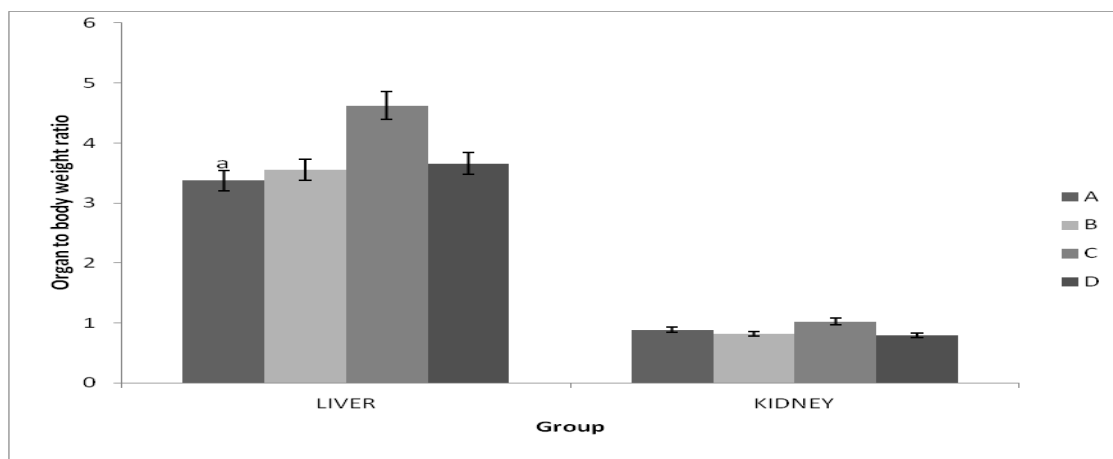


Figure 2: Organ to Body Weight Ratio of rats exposed to various forms of water

Values are Mean $\pm$ SEM. Values are Mean $\pm$ SEM. Bars with different letter have values that are significantly different ($p < 0.05$).

Key: A: Animals exposed to tap water. B: Animals exposed to *Moringa oleifera* seed powder-treated Asa River water. C: Animals exposed to Asa River water. D: Animals exposed to *Moringa oleifera* seed powder treated tap water

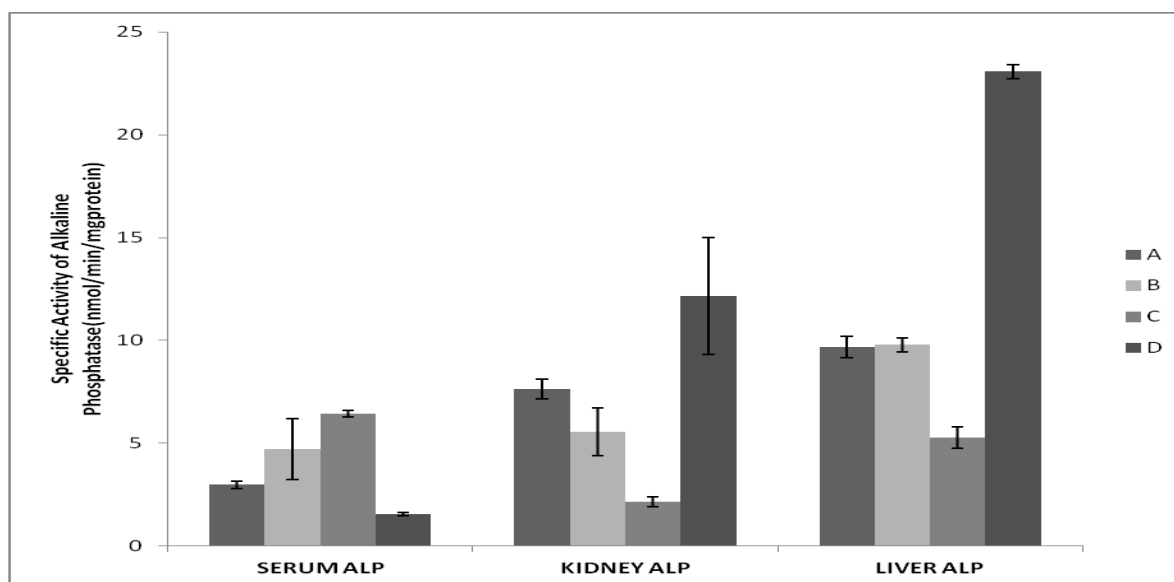


Figure 3: Specific Activity of Alkaline Phosphatase of the Rats exposed to various forms of water

Values are Mean $\pm$ SEM. Values are Mean $\pm$ SEM. Bars with different letter have values that are significantly different ($p < 0.05$).

Key: A: Animals exposed to tap water. B: Animals exposed to *Moringa oleifera* seed powder-treated Asa River water. C: Animals exposed to Asa River water. D: Animals exposed to *Moringa oleifera* seed powder treated tap water

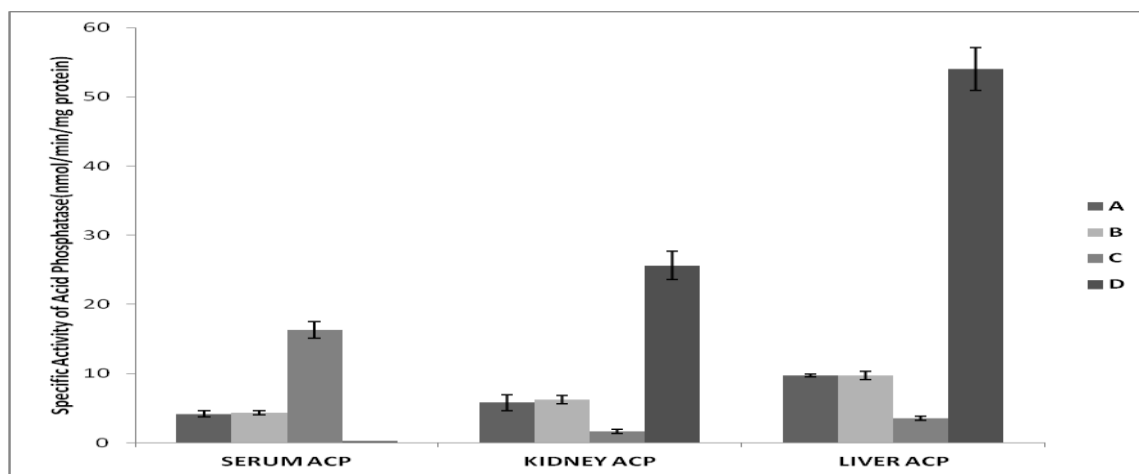


Figure 4: Specific activity of acid phosphatase of the rats exposed to various forms of water

Values are Mean $\pm$ SEM. Values are Mean $\pm$ SEM. Bars with different letter have values that are significantly different ($p < 0.05$).

Key: A: Animals exposed to tap water. B: Animals exposed to *Moringa oleifera* seed powder-treated Asa River water. C: Animals exposed to Asa River water. D: Animals exposed to *Moringa oleifera* seed powder treated tap water

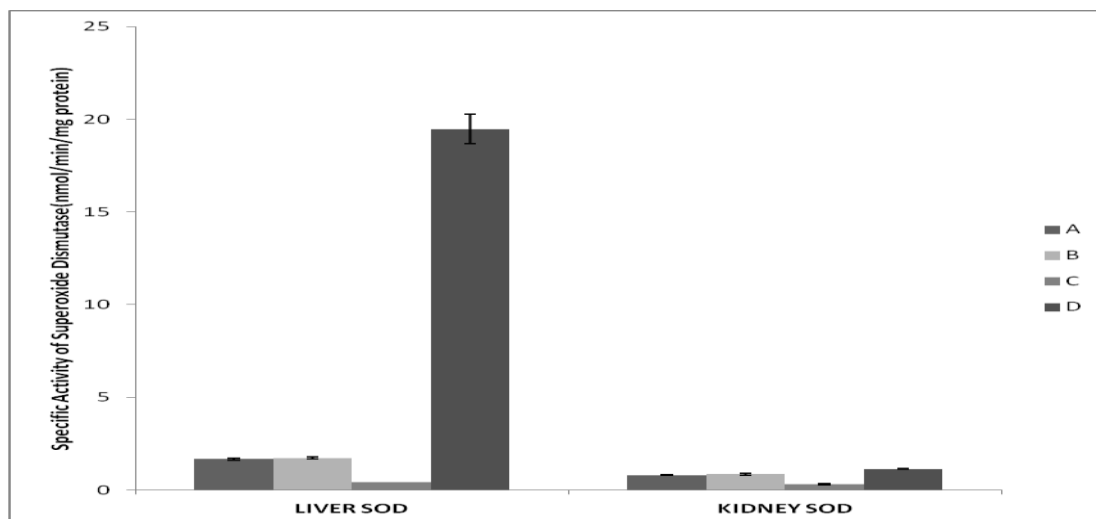


Figure 5: Specific Activities of Superoxide Dismutase of the Rats exposed to various forms of water.

Values are Mean $\pm$ SEM. Values are Mean $\pm$ SEM. Bars with different letter have values that are significantly different ($p < 0.05$).

Key: A: Animals exposed to tap water. B: Animals exposed to *Moringa oleifera* seed powder-treated Asa River water. C: Animals exposed to Asa River water. D: Animals exposed to *Moringa oleifera* seed powder treated tap water

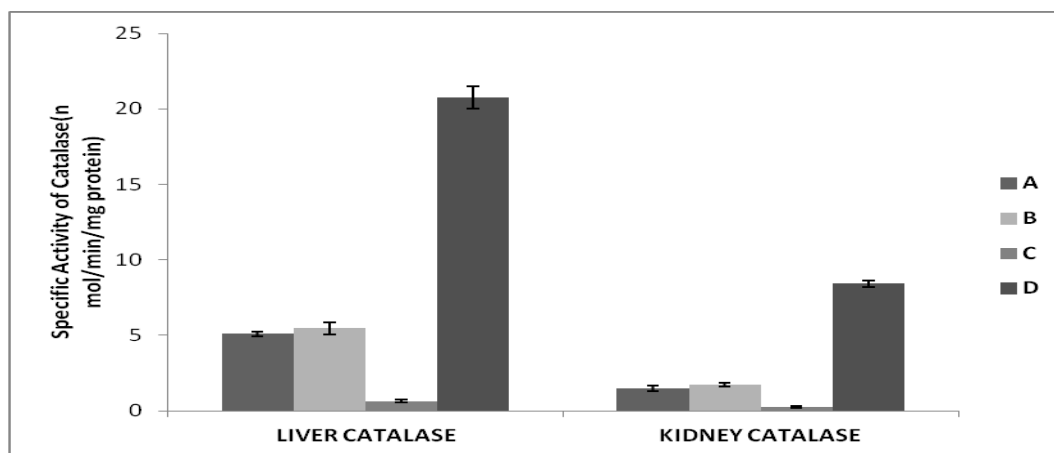


Figure 6: Specific activities of catalase of the rats exposed to various forms of water

Values are Mean $\pm$ SEM. Values are Mean $\pm$ SEM. Bars with different letter have values that are significantly different ($p < 0.05$).

Key: A: Animals exposed to tap water. B: Animals exposed to *Moringa oleifera* seed powder-treated Asa River water. C: Animals exposed to Asa River water. D: Animals exposed to *Moringa oleifera* seed powder treated tap water

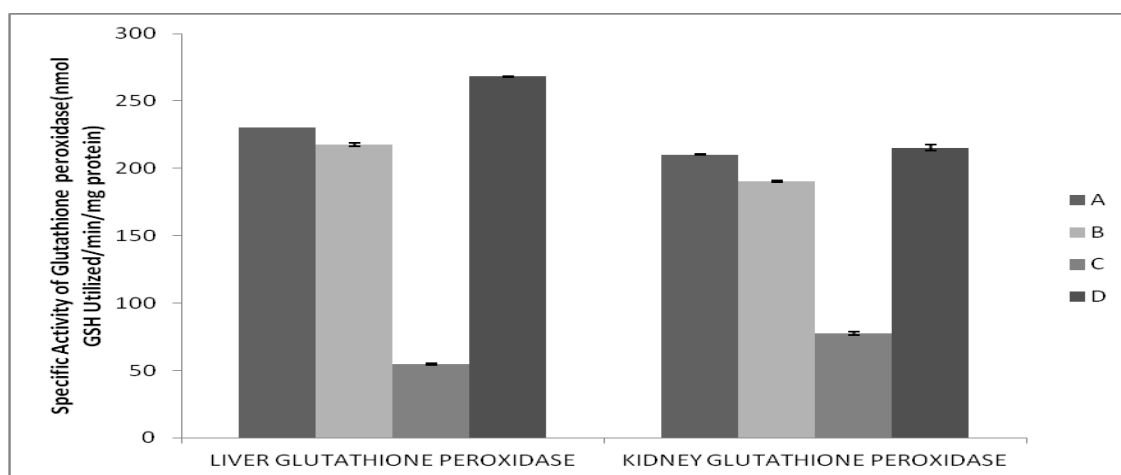


Figure 7: Specific Activities of Glutathione Peroxidase of the Rats exposed to various forms of water

Values are Mean $\pm$ SEM. Values are Mean $\pm$ SEM. Bars with different letter have values that are significantly different ($p < 0.05$).

Key: A: Animals exposed to tap water. B: Animals exposed to *Moringa oleifera* seed powder-treated Asa River water. C: Animals exposed to Asa River water. D: Animals exposed to *Moringa oleifera* seed powder treated tap water

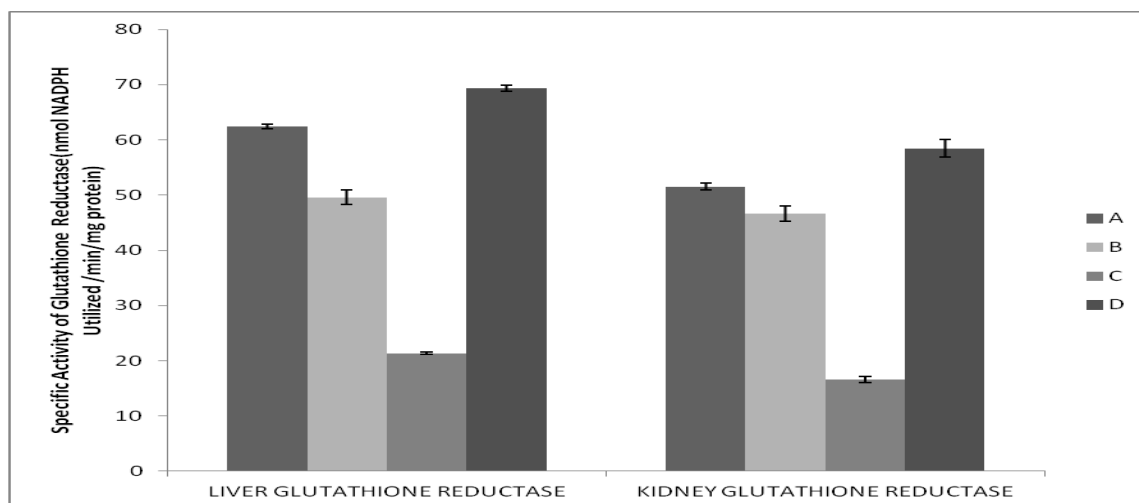


Figure 8: Specific Activities of Glutathione reductase of the Rats exposed to various forms of water.

Values are Mean $\pm$ SEM. Values are Mean $\pm$ SEM. Bars with different letter have values that are significantly different ($p < 0.05$).

Key: A: Animals exposed to tap water. B: Animals exposed to *Moringa oleifera* seed powder-treated Asa River water. C: Animals exposed to Asa River water. D: Animals exposed to *Moringa oleifera* seed powder treated tap water

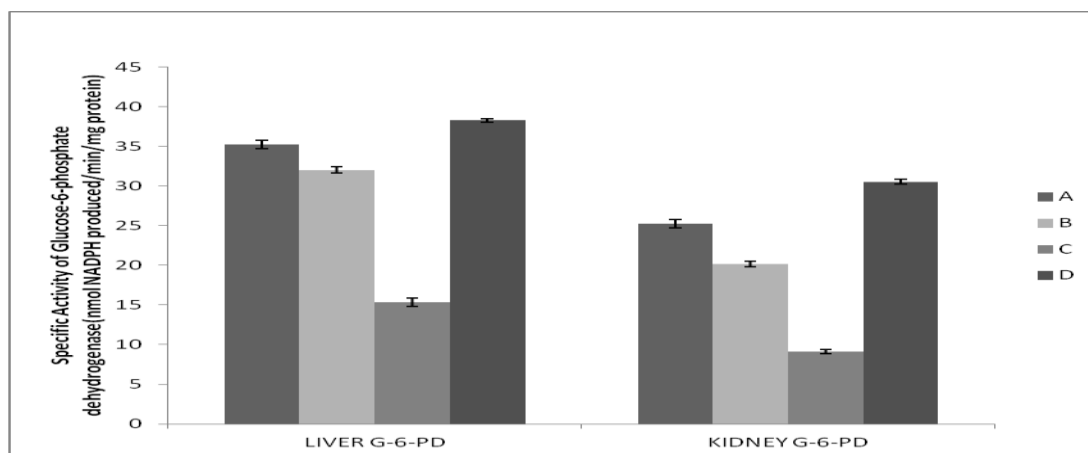


Figure 9: Specific Activities of Glucose-6-phosphate dehydrogenase of the Rats exposed to various forms of water.

Values are Mean $\pm$ SEM. Values are Mean $\pm$ SEM. Bars with different letter have values that are significantly different ($p < 0.05$).

Key: A: Animals exposed to tap water. B: Animals exposed to *Moringa oleifera* seed powder-treated Asa River water. C: Animals exposed to Asa River water. D: Animals exposed to *Moringa oleifera* seed powder treated tap water

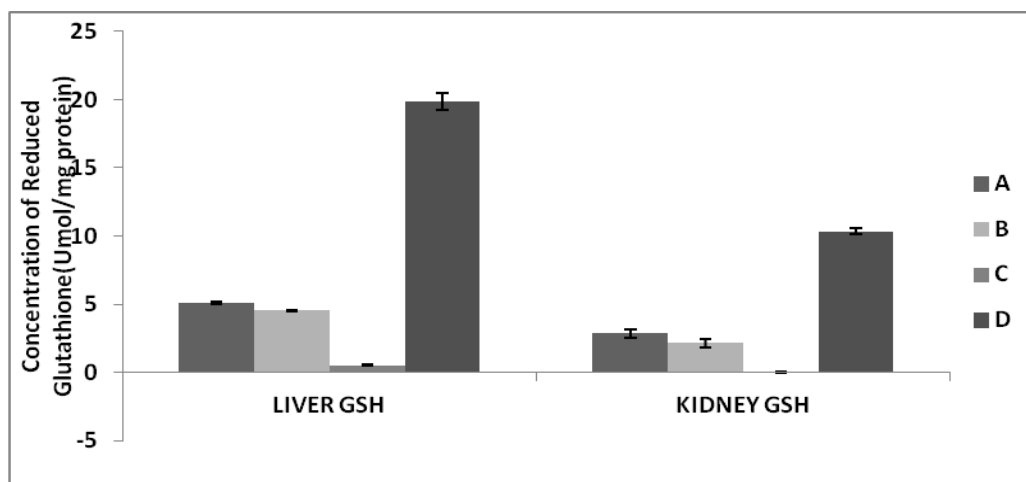


Figure 10: Concentration of Reduced Glutathione (GSH) of the Rats exposed to various forms of water.

Values are Mean $\pm$ SEM. Values are Mean $\pm$ SEM. Bars with different letter have values that are significantly different ($p < 0.05$).

Key: A: Animals exposed to tap water. B: Animals exposed to *Moringa oleifera* seed powder-treated Asa River water. C: Animals exposed to Asa River water. D: Animals exposed to *Moringa oleifera* seed powder treated tap water

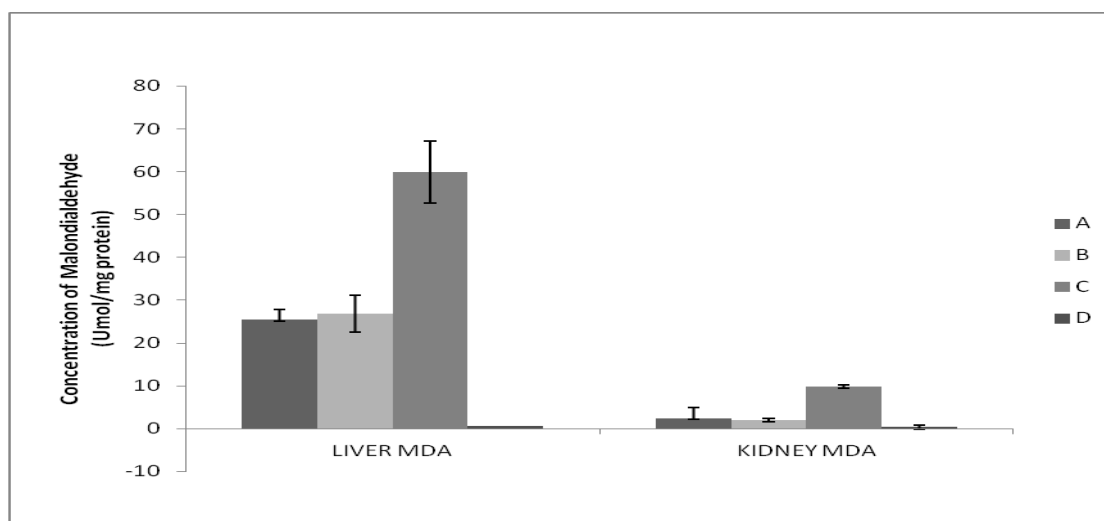


Figure 11: Concentrations of Malondialdehyde (MDA) of Rats exposed to various forms of water

Values are Mean $\pm$ SEM. Values are Mean $\pm$ SEM. Bars with different letter have values that are significantly different ($p < 0.05$).

Key: A: Animals exposed to tap water. B: Animals exposed to *Moringa oleifera* seed powder-treated Asa River water. C: Animals exposed to Asa River water. D: Animals exposed to *Moringa oleifera* seed powder treated tap water

Asa River Water being classified as highly turbid (Lea, 2010). The turbid nature of Asa River Water may arise from the sediments occasioned by the effluents received from the nearby industries, domestic wastes and farming activities. The higher figures in respect of the pH and total solids (TS) than those of the WHO limits further support the unacceptability of the Asa River water for human consumption and may pose threat to public health. Consequently, the Asa River Water by virtue of the findings earn the status of “being polluted”.

The polluted status of Asa River Water is also supported by the striking evidence of the presence of heavy metals which include in particular mercury, cadmium, and arsenite which are 28-folds, 1.5 folds and 14-folds respectively higher than the values stipulated by the WHO standards. This findings may affirm heavy metal toxicity of the Asa River Water if consumed. Bohara et al. (2008) reported that heavy metals possess the ability to generate reactive oxygen species (ROS) which can cause oxidative stress and lipid peroxidation and inactivation of antioxidant enzymes. Consequently, the possibility of the consumption of Asa River Water inducing oxidative stress and being unfit for human consumption is further emphasised.

The liver and the kidney respectively perform the functions of detoxifying xenobiotics and excreting ingested toxic products (Jacob and Francoise, 1989). The evidence in this study in respect of the significant increase in the liver to body weight and kidney to body weight ratios consequent upon the consumption of Asa River Water by the experimental animals (Figure 2) is therefore not surprising. It is considered that the tissues are overwhelmed with the oxidative stress imposed by the constituents including heavy metals of Asa River Water and therefore manifests as hypertrophy of the liver and kidney. Similar increase in liver to body weight has earlier been reported by Guanghe et al (2005) and Li et al. (2006). However, the observation with respect to the tissues of rats exposed to *Moringa oleifera* seed powder-treated Asa River water exhibiting no significant difference with the tissues of the control animals is indicative of the preventive potential of *Moringa oleifera* seed powder on oxidative stress.

The consequence of oxidative stress can manifest as changes in key biochemical parameters including enzymes activities. For instance, ALP is used to assess the integrity of the plasma membrane (Akanji et al., 1993), hence any alteration in ALP activity is indicative of likely damage to the external boundary of the cells. Our findings in the current study with respect to Asa River Water to the significant ($p < 0.05$) reduction in the specific activity of ALP in the liver and kidney of rat (Figure 3) of rat exposed would arise from the oxidative stress imposed on the tissues by the water and resulting in their damages with leakages of ALP into the serum. Similarly, a significant ($p < 0.05$) decrease in the specific activity of liver and kidney ACP, accompanied by corresponding significant ($p < 0.05$) increase in that of serum ACP in rats exposed to Asa River water (Figure 4) could be attributed to disruption of membrane structure by Asa River-water-induced oxidative stress. This induced oxidative stress resulting from increased ROS generation could be as result of the adverse effect of high heavy metals concentration of the Asa River water. However, the specific activities of the liver and kidney ACP of the *Moringa oleifera* seed powder-treated Asa River water exposed rats which showed no significant difference from those of the Normal control could suggest the Preventive Activity of the *Moringa oleifera* seed powder on Asa River-water-induced oxidative stress (Figure 4) while observed the significant ($p > 0.05$) increase in the specific activities of the liver and kidney ACP in the rats exposed to *Moringa oleifera* seed powder-treated tap water may suggest the ability of the *Moringa oleifera* seed powder to restore the ACP.

The enzymatic antioxidant system, which include SOD, CAT, GSH-Px, GSH-Red, as well as G-6-PD, plays a coordinated role in the preventive damage by ROS (Ajiboye et al., 2010). The specific activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase (Figures 5- 9) were markedly decreased in Asa River water exposed rats. This result is indifferent with earlier report of Jadhav et al. (2007) who demonstrated oxidative stress and decreased antioxidant status by

measuring lipid peroxidation and activities of GSH, catalase and glutathione peroxidase following consumption of ground water containing mixture of metals. Superoxide radicals by themselves or after their transformation to hydrogen peroxide (H_2O_2) caused oxidation of the cysteine in the enzyme and decreased the SOD activity (Dimitrova *et al.*, 1994).

The degradation of H_2O_2 , a potent oxidant at high cellular concentration, is effected by CAT. The decrease in the activities of enzymatic antioxidants in Asa River water treated rats could be due to their utilization in countering the effect against increased oxidative stress. However, specific activities of the antioxidant markers in rats exposed to *Moringa oleifera* seed powder-treated Asa River water which showed no significant difference from those of Normal control rats could be due to ability of *Moringa oleifera* seed powder to prevent the Asa River-water-induced oxidative stress. This further buttressed the preventive activity of *Moringa oleifera* seed powder on Asa River-water-induced oxidative stress. The observed significant ($p > 0.05$) increase in the specific activities of antioxidant markers in *Moringa oleifera* seed powder-treated tap water, relative to control may suggest the ability of the *Moringa oleifera* seed powder to boost the antioxidant systems and this may further buttress the preventive activity of the *Moringa oleifera* seed powder on Asa River-water-induced oxidative stress.

The non-enzymatic antioxidant system complements the activity of the enzymatic antioxidants system in preventing cells from excessive oxidative stress by acting as free radical scavengers (Ajiboye *et al.*, 2010). GSH scavenges free radicals and ROS thus protecting lipids from the attack of electrophilic compounds by reacting with their electrophilic centre with the aid of its thiol (-SH) group (Sigh and Lahluwali, 2003). The GSH plays a crucial role in protecting the cells from oxidative damage (Guoyao *et al.*, 2004). In the results of study, liver and kidney GSH (Figure 10) concentrations were significantly ($p < 0.05$) decreased after exposure of the rats to Asa River water. This significant ($p < 0.05$) decrease in GSH concentrations under the present

experimental model may suggest its over utilization to challenge the prevailing oxidative stress under the influence of ROS generated from Asa River-water-induced oxidative stress. The result of this study is indifferent with the earlier report of Bray and Taylor (1993) which reported a significant decrease of liver and kidney GSH concentrations were during increased oxidative stress. However, the concentrations of liver and kidney GSH in *Moringa oleifera* seed powder-treated Asa River water exposed rats which showed no significant difference from those of the Normal control rats could suggest the preventive activity of *Moringa oleifera* seed powder on Asa River-water-induced oxidative stress (Figure 10). The concentrations of liver and kidney GSH (Figure 10) which significantly ($p > 0.05$) increased in rats exposed to *Moringa oleifera* seed powder-treated tap water could suggest the ability of *Moringa oleifera* seed powder to induce GSH production. This could further buttress the preventive activity of *Moringa oleifera* seed powder on Asa River-water-induced oxidative stress.

Depletion of GSH-Px and GSH-Red has a direct relationship to GSH, as these enzymes maintain high intracellular GSH:GSSG ratio and thus protect cellular molecules against oxidative attack (Kozar *et al.*, 2003). Therefore, the protection of non-enzymatic antioxidant system by *Moringa oleifera* seed powder treated water suggests its preventive activity on Asa River-water-induced oxidative stress.

Evidence suggests that various enzymatic and non enzymatic systems have been developed by the cell to cope with the ROS and other free radicals. However, when a condition of oxidative stress is established, the defense capacities against ROS become insufficient (Halliwell and Gutteridge, 2000). ROS attack cellular components involving polyunsaturated fatty acid residues of phospholipids, which are extremely sensitive to oxidation. Once formed, peroxy radicals ($ROO\cdot$) can be rearranged via a cyclization reaction to endoperoxides (precursors of MDA) with the final product of the peroxidation process being MDA (Marnett, 1999). Administration of Asa River water resulted in significant ($p > 0.05$) increase in MDA level in treated animals. This result is consistent

with previous study on oxidative damage induced in liver, kidney and spleen of mice treated with landfill leachate (Li *et al.*, 2006). This significant ($p > 0.05$) increase in MDA concentration (Figure 11) of Asa River water exposed animals further indicated that peroxidation of polyunsaturated fatty acids present on the plasma membrane of the hepatocytes have occurred. This is consistent with past studies which demonstrated increased lipid peroxidation or decrease in antioxidant defence mechanism under heavy metals induced oxidative stress (Bokara *et al.*, 2008). This also indicates the disruption of plasma membrane which in turn led to the loss of membrane bound enzyme (ALP) from the hepatocytes to the serum as a consequence of peroxidation of unsaturated fatty acids. However, the concentration of MDA in rats exposed to *Moringa oleifera* seed powder-treated Asa River water which showed no significant difference from those of Normal control could suggest the ability of *Moringa oleifera* seed powder to prevent Asa River-water-induced oxidative stress. The significant ($p < 0.05$) decrease in MDA concentration (Figure 11) observed in rats exposed to *Moringa oleifera* seed powder treated tap water may be due to the ability of the seed powder to reduce lipid peroxidation. This further buttress the preventive activity of *Moringa oleifera* seed powder on Asa River-water-induced oxidative stress.

Histological changes in tissues are late manifestation of a chemical, physical, mechanical or inflammatory assault on the tissue (Adesokan, 2007). In the histopathological results (Plates 1 to 8) of this study, the degenerated histoarchitectural structures of the liver and kidney (Plate 3 and Plate 7 respectively) observed in Asa-River-water exposed rats could be due to assaults of Asa River-water-induced oxidative stress.

The liver histopathological result of this study is in support of earlier report of Mazumdar (2005) reported chronic intake of contaminated water, induces oxidative stress in liver, resulting in hepatomegaly, characterized by fatty degeneration and progressive reduction of hepatic glutathione and enzymes of antioxidative defense associated with lipid peroxidation. However,

The administration of *Moringa oleifera* treated Asa River water revealed a normal histoarchitectural structure of the liver and kidney (Plates 2 and 6 respectively).

Conclusion

The data obtained from this study show that *Moringa oleifera* seed powder could be an alternative and safe coagulant for treatment of polluted water such as the Asa River water and also capable of preventing oxidative stress that can be induced by the untreated water. This is evident in : The physicochemical characteristics of the Asa River water (Table 1); restoration of toxicological markers (ALP and ACP) by *Moringa oleifera* seed powder as evident in Figure 3 and Figure 4; protection of antioxidant system by *Moringa oleifera* seed powder as evident in Figures 5 – 10; reduction of lipid peroxidation by *Moringa oleifera* seed powder as evident in Figure 11 and protection of histoarchitectural structure by *Moringa oleifera* seed powder as evident in Plate 1 – Plate 8.

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