Biochemical Evaluation of Oxidative Damage Induced by Leachate Contaminated Groundwater on Selected Tissues of Rats

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Citation

O Adeyemi, O Oleyede, A Oladiji. *Biochemical Evaluation of Oxidative Damage Induced by Leachate Contaminated Groundwater on Selected Tissues of Rats*. The Internet Journal of Toxicology. 2007 Volume 4 Number 2.

Abstract

Some parameters of oxidative stress: malondialdehyde, reduced glutathione, catalase and superoxide dismutase were determined in rats placed on leachate-contaminated groundwater over a period of sixty five days. Significant increase in malondialdehyde (MDA) concentrations is observed in test rats relative to control ($p<0.05$). In particular, the concentration of MDA in the liver of rats placed on the first well water (14.00±0.5 nmol/mg tissue) was significantly ($p<0.05$) higher than that of control (12.47±1.2). Conversely, reduced glutathione (GSH) concentration, catalase activity and superoxide dismutase (SOD) activity were significantly lower in test rats relative to the control group ($p<0.05$). Particularly, the activity of SOD in the liver of rats (0.58±0.01 Unit/mg protein) placed on first well water was significantly ($p<0.05$) lower than that of rats placed on tapwater (0.90±0.02). From the result, it is probable that consumption of leachate-contaminated groundwater may induce oxidative stress by causing membrane lipid peroxidation.

INTRODUCTION

Depending on climatological, geographical and hydrological conditions at site, the amount of leachate generated and its characters vary [1]. Several factors have been identified to affect the composition of landfill leachate. These factors include the type of waste materials put into the landfill, characteristics of precipitation entering the landfill, and landfill conditions such as the pH, temperature, moisture, age and climate [2]. Generally, leachates are composed of heavy metals, organic and inorganic components that are sources of reactive oxygen species (ROS) [3]. ROS generate free radicals in exposed tissues [4]. Free radicals are released in the body from detoxification of drugs, artificial food colouring and flavouring, smog, preservatives in processed foods, alcohol, cigarette smoke, chlorinated water, pesticides, cleaning fluids, heavy metals and assorted chemicals such as solvent traces found in processed foods and aromatic hydrocarbons such as benzene and naphthalene which are major components of landfill leachate [5].

Reactive oxygen species has been implicated in the development of cardiovascular and cerebrovascular diseases by causing membrane lipid peroxides and toxic malondialdehyde (MDA) can damage proteins and DNA and have also been implicated in carcinogenesis [6]. Oxidation of lipids modifies membranes and impairs their function. Fluidity is decreased, membrane bound enzymes and receptors are inactivated, red blood cells are damaged and endothelial cells are injured, increasing blood vessels fragility [7].

However, as a means of self defense, nature has made available some substances called antioxidants to decompose peroxides, inactivate metals, scavenge free radicals and hinder lipid peroxidation [8]. These antioxidants can be enzymes (SOD, catalase, glutathione peroxidase and glutathione-s-transferase) and non-enzyme (glutathione, ascorbic acid, ubiquinones, and ?-tocopherol etc) [9]. Lower levels of these antioxidants in the body portend a condition of oxidative stress [10].

Previous study [11] had shown that leachate-contaminated groundwater around Odo Iya Alaro landfill in Lagos, the former capital of Nigeria, contains heavy metals such as lead (Pb) and cadmium (Cd) which have been implicated in oxidative damage. The present study examined the probable oxidative damage to tissues arising from the consumption of leachate-contaminated groundwater around Odo Iya Alaro landfill.

MATERIALS AND METHODS

Chemicals and solvents are of analytical grade and are products of Sigma-Aldrich Inc, St. Louis, U.S.A. Solid wastes were collected at various parts from a landfill located in Ojota, Lagos, Nigeria. Leachate was simulated from the wastes follows the method described by [12].

Sixty (60) Albino rats (Rattus novergicus) were obtained from the Small Animal Holding of the Department of Biochemistry University of Ilorin, Ilorin, Nigeria. These rats were fed ad libitum with commercial feeds obtained from Livinco feeds, Jubilee road, Ikare Akoko, Ondo State, Nigeria. The experimental animals were grouped into two sets of 30 rats each, each set of rats were kept inside a wooden cage assigned into six (6) groups of five (5) animals each. The first two groups of rats were placed on tap water and simulated leachate samples respectively. The third and fourth groups were placed on water samples obtained from wells located at a distance of about 1km and 1.5km respectively from Odo Iya Alaro landfill. The remaining two groups of rats were placed on water samples obtained from boreholes located at a distance of about 1km and 1.5km respectively from the landfill. The feeding exercise lasted over a period of 65days, a long term standard for rats [13], preceded by 10 days acclimatization period. The second set of rats represents a replicate of the first set.

At the end of the 65days the rats were sacrificed by anaesthetizing in a jar containing cotton wool soaked in diethylether. The jugular veins were cut and blood samples were collected first in heparinized bottles and then in stopped plastic tubes. The latter was centrifuged at a speed of 10, 000 RCF for 5minutes. The serum was collected and stored at -8?C until required for use. Selected tissues; liver, kidney, stomach and colon were isolated, homogenized in 0.25M sucrose solution and stored at -8oC until required for use.

The protein content in the tissue homogenates were determined using the Biuret method of Gornal[14]. Cupric ions in alkaline solution form a purple coloured complex with any compound containing repeated-CONH-links such as proteins. The colour intensity which is a measure of the protein content of a sample is measured spectrophotometrically at 540nm. MDA determination was based on method described by Bird[15]. MDA reacts with thiobarbituric acid to give a red complex which is measured spectrophotometrically at 535nm. The method described by Jollow[16] was used to determine reduced glutathione (GSH) concentration. The absorbance was read at 412nm. Catalase activity was determined according to the method of Sinha[17]. The method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2 with the formation of perchloric acid as an unstable intermediate. The chromic acetate was then measured spectrophotometrically at 570nm. The activity of superoxide dismutase (SOD) was determined by the method of Misra and Fridovich[18]. This method is based on the ability of SOD to inhibit the autoxidation of epinephrine at pH 10.2. The absorbance was measured at 480nm.

STATISTICAL ANALYSIS

The two way analysis of variance ANOVA; Duncan's multiple range test (DMRT) was the statistical analysis used. $p<0.05$ was regarded as significance.

RESULTS AND DISCUSSION

The MDA concentrations of tissues of rats placed on leachate-contaminated groundwater are presented in Table 1. Generally, the MDA concentration in tissues of rats placed on leachate-contaminated groundwater is significantly ($p<0.05$) higher than that of control rats. Of all the tissues studied, the MDA concentration in the colon and serum of the rats placed on simulated leachate was about three and two folds respectively, that of rats placed on tapwater. Increased MDA levels in tissues and serum is closely related with conditions that exert oxidative stress[19]. The observation in the present study may be attributable to the presence of heavy metals such as Pb and Cd in the groundwater samples, which are capable of inducing oxidative stress.

Table 2 shows the concentration of reduced glutathione in the tissues of rats placed on leachate-contaminated groundwater over a period of 65 days. GSH concentrations in the tissues of rats placed on leachate-contaminated groundwater was significantly ($p<0.05$) lower than that of control rats. The reduction fell between the range of 1/3 in the case of liver and kidney, and ½ in the colon and stomach of rats placed on simulate leachate. Depletion of GSH concentration in tissues decreases cellular antioxidant defence ability and increase oxidative stress[20]. Heavy metals and microorganisms release free radicals that are able to abstract electrons from oxidizable substrates (e.g. GSH) of tissues thereby exposing the tissue to oxidative damage[21]. Decreased GSH level as observed in the tissues of rats

maintained on daily consumption of leachate-contaminated groundwater samples may be partly due to the presence of heavy metals and bacteria in the groundwater samples.

The activity of catalase in tissues of rats placed on leachate-contaminated groundwater for 65 days is presented in Table 3. Catalase activity in tissues of rat placed on leachate-contaminated groundwater was significantly lower ($p<0.05$) than that of control animals placed on tap water sample. Generally, the reduction was about ½ in the tissues of the rats placed on simulated leachate relative to those of the rats placed on tapwater. The decrease in the activity of catalase in tissue of test animals, as observed in this study lends credence to the submission that pollutants of the leachate-contaminated groundwater samples may possess the ability of inducing oxidative damage to tissues of rats.

Table 4 shows the activity of superoxide dismutase (SOD) in the tissues of rats placed on leachate-contaminated groundwater over a period 65 days. A general significant ($p<0.05$) decrease in the activity of SOD was observed in the tissues of rats placed on leachate and leachate-contaminated groundwater samples relative to those of rats placed on tap water sample. The reduction fell between the range of $1/_3$ in the kidney, colon and stomach, and ½ in the liver of rats placed on simulated leachate relative to the control rats. Pb and Cd had been reported to generate free radicals and cause membrane lipid peroxidation [22]. The observed decrease in SOD activity in tissues of experimental rats could be due to oxidative stress induced by pollutants, especially Pb and Cd, in the groundwater samples.

On the whole, biochemical evidences from the present study suggest that the consumption of leachate-contaminated groundwater in Ojota, Lagos, Nigeria could lead to oxidative damage to tissues and should therefore be discouraged.

Figure 1

Table 1: Effect of leachate-contaminated groundwater on MDA concentration (nmol/mg tissue) of selected tissues of rats.

Group	Liver	Kidney	Colon	Stomach	serum
Tapwater	12.47±1.2^a	17.91±1.4^a	3.17±0.3^a	5.67±0.5^a	1.00±0.2^a
Leachate	15.99±1.3^b	25.15±2.1^b	10.54±1.3^b	8.04±0.8^b	2.20±0.5^b
Well (1Km)	14.00±0.5^c	21.11±1.1^c	8.84±1.0^c	7.48±0.4^c	1.96±0.2^c
Well (1.5Km)	13.47±0.6^c	20.07±0.8^c	6.40±0.8^d	7.41±0.5^c	1.66±0.2^d
Borehole (1Km)	13.45±0.7^c	17.97±0.6^d	4.54±0.4^e	5.9±0.3^d	1.39±0.5^e
Borehole (1.5Km)	13.17±1.0^c	17.88±0.8^d	4.24±0.6^e	5.87±0.2^d	1.27±0.5^e

Results are means of 10 determinations ± SEM. Values in the same column with different superscripts are significantly different ($p<0.05$)

Figure 2

Table 2: Effect of leachate-contaminated groundwater on GSH concentration (µg/mg tissue) of selected tissues of rats.

Group	Liver	Kidney	Colon	Stomach
Tapwater	1.1±0.10^a	1.12±0.30^a	0.57±0.05^a	0.53±0.01^a
Leachate	0.38±0.04^b	0.37±0.06^b	0.2±0.01^b	0.22±0.01^b
Well (1Km)	0.66±0.10^c	0.6±0.03^c	0.36±0.01^c	0.34±0.02^c
Well (1.5Km)	0.69±0.05^c	0.64±0.02^c	0.39±0.03^c	0.37±0.02^c
Borehole (1Km)	0.93±0.05^d	0.9±0.10^d	0.48±0.01^d	0.48±0.02^d
Borehole (1.5Km)	0.98±0.06^d	0.96±0.09^d	0.49±0.01^d	0.50±0.01^d

Results are means of 10 determinations ± SEM. Values in the same column with different superscripts are significantly different ($p<0.05$)

Figure 3

Table 3: Effect of leachate-contaminated groundwater on specific activity of catalase (µmole of HO decomposed/min/mg protein) in selected tissues of rats.

Group	Liver	Kidney	Colon	Stomach
Tapwater	0.60±0.01^a	1.04±0.06^a	0.30±0.01^a	0.33±0.01^a
Leachate	0.27±0.01^b	0.47±0.01^b	0.16±0.01^b	0.19±0.01^b
Well (1Km)	0.46±0.02^c	0.73±0.02^c	0.20±0.01^c	0.22±0.01^c
Well (1.5Km)	0.48±0.01^c	0.74±0.01^c	0.21±0.01^c	0.23±0.01^c
Borehole (1Km)	0.56±0.01^d	0.86±0.03^d	0.27±0.01^d	0.27±0.01^d
Borehole (1.5Km)	0.55±0.02^d	0.90±0.02^d	0.27±0.01^d	0.28±0.01^d

Results are means of 10 determinations ± SEM. Values in the same column with different superscripts are significantly different ($p<0.05$).

Figure 4

Table 4: Effect of leachate-contaminated groundwater on specific activity of SOD (Unit/mg protein) in selected tissues of rats.

Group	Liver	Kidney	Colon	Stomach
Tapwater	0.90±0.02^a	1.04±0.02^a	0.43±0.01^a	0.54±0.02^a
Leachate	0.30±0.01^b	0.38±0.01^b	0.12±0.00^b	0.17±0.00^b
Well (1Km)	0.58±0.01^c	0.5±0.02^c	0.2±0.00^c	0.23±0.01^c
Well (1.5Km)	0.62±0.01^d	0.55±0.01^d	0.22±0.01^d	0.25±0.01^d
Borehole (1Km)	0.70±0.02^e	0.6±0.01^e	0.35±0.01^e	0.39±0.01^e
Borehole (1.5Km)	0.78±0.02^f	0.69±0.03^f	0.38±0.01^f	0.43±0.01^f

Results are means of 10 determinations ± SEM. Values in the same column with different superscripts are significantly different ($p<0.05$).

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