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African land snail *Achatina marginatus*, as bioindicator of environmental pollution

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Abstract. Activity of xanthine oxidase, levels of lipid peroxidation and ascorbic acid were studied in African land snail (*Achatina marginatus*) from two sites with different pollution potentials. Lipid peroxidation was significantly higher in the snails from the polluted site compared to the value obtained from the same species from the control site. Similarly, the activity of xanthine oxidase in the snails from the polluted site was significantly higher when matched with the value obtained for the corresponding species from the control site. Conversely, ascorbic acid content in the snails from the polluted site was significantly lower compared to the value obtained for the snails from the control site. The data presented here suggest that the upregulation of the activity of xanthine oxidase level of lipid peroxidation and the corresponding reduction in ascorbic acid content is related to oxidative stress in this species and could therefore possibly make it a bioindicator of environmental pollution.

Key words: Ascorbic acid, level of lipid peroxidation, petroleum pollution, xanthine oxidase, snail.

Introduction

Nigeria is one of the major petroleum producing countries of the world and the environmental impact associated with its exploration and exploitation has been a popular area of experimental research in the last three decades (Odjegba & Sadiq 2002). The environment of Warri is highly polluted due to enormous oil exploration activities coupled with the presence of petroleum refinery and petrochemical complex which discharge effluent into the surrounding ecosystem (Egborge & Benka-Coker 1986, Achuba et al. 2005).

Xanthine oxidase (xanthine: oxygen oxidoreductase EC 1.2.32, XO) is a family of molybdenum hydroxylases iron sulphur flavoprotein involved in the degradation of adenosine triphosphate to uric acid by converting hypoxanthine via xanthine into uric acid (Xia et al. 1999). The ability of xanthine oxidase to generate superoxide anion and hydrogen peroxide in the presence of molecular oxygen, hypoxanthine and xanthine has been documented (Fields et al. 1996). A general pathway of toxicity for many pollutants is mediated by the enhancement of intracellular reactive species, which modulate the occurrence

of cell damage (Regoli et al. 2002, 2003, Sioutas et al. 2005) via initiation and propagation of lipid peroxidation (Gutteridge 1995). Lipid peroxidation is a complex process in which poly-unsaturated fatty acids in biological membrane system undergo changes by chain reactions and form lipid hydroperoxides which decompose double bonds of unsaturated fatty acids and disrupt membrane lipid (Gutteridge 1995, Isamah et al. 2003). Ascorbic acid, a non-enzyme anti-oxidant has a role in defense against oxidative stress (Kilts 1997, Buettner & Jurkiewicz 1996, Puskas et al. 2000, Smirnoff & Wheeler 2000).

Terrestrial invertebrates are often used to monitor air and soil pollution (Dallinger 1994). This is because they have the ability to accumulate both organic and inorganic chemicals of diverse origin and respond to them both at organism and cellular levels (Berger & Dallinger 1993, Dallinger 1996, Gomot de Vaufleurg & Pihari 2000, Gomot de Vaufleury & Kerhvas 2000, Snyman et al. 2000, Beeby & Richmond 2002, 2003, Viard et al. 2004, Ragoli et al. 2005). This paper reports on the activity of xanthine oxidase, lipid peroxidation and ascorbic acid in African snail, *Achatina marginatus*, from two environments with different pollution potentials.

Materials and methods

120 specimens of African land snail, *Achatina marginatus,* (60 from the polluted area and 60 from the non-polluted area), having an average net weight 35±3.6g and length 6±2.3cm were obtained from Ekpan /Warri, with a history of petroleum pollution and from Abraka, which serve as control. The snail species was duly identified by the department of Zoology, Delta State University, Abraka. The collected individuals were sorted and those with signs of disease were discarded and the healthy ones were kept in a cool environment with food until they were dissected and the foot muscle was extracted for analysis.

The shell of the snail was removed and the foot muscle was isolated under ice (4^0C). From the isolated organs, 0.5g were separated and homogenized with 10mL of ice-cold 0.05M Phosphate buffer, pH 7.4 and butylated hydroxy toluene (BTH) using an MSE blender immersed in ice. The homogenate was filtered with double layered cheese cloth and the filtrate was centrifuged at 7000g for 20minutes (4^0C). The supernatant (S_1) was used for the determination of lipid peroxidation (Gutteridge & Wilkins 1982). The process was repeated using another four snails to obtain a total of five determinations.

Xanthine oxidase was measured with a similar (S_1) fraction and its assay is based on the ability to catalyze the conversion of methylene blue to the reduced colourless form (ADAC, 1984)

Each muscle sample, 2.0g was mixed with 20mL of 0.05M phosphate buffer, pH 7.4 and then acidified with 5% metaphosphoric acid (5 volume of sample plus 1 volume of acid) and ascorbic acid content determined with 2,6-dichlorophenol-indophenol (DCIP) (Plummer 1978).

Comparisons between polluted sites and control were made by using Student's T-test and differences at $P < 0.05$ were considered as significant. The results were expressed as mean $\pm$ SE.

Results and discussion

The activiy of xanthine oxidase and lipid peroxidation were significantly higher (t test, $P<0.05$) in the snail from

the polluted site as compared with the corresponding species from the control site (Table 1).

The upregulation of xanthine oxidase activity in the snail from the polluted site could be an adaptive mechanism to prevent the accumulation of toxic reactive oxygen intermediates. A wealth of information is available confirming that xanthine oxidase is involved in the metabolism of heterocyclic and polycyclic aromatic hydrocarbon (Panoutsopoulos & Beedham 2004, Panoutsopoulos et al. 2004). A number of enzymes such as xanthine oxidase produce superoxide anion (Fridovich 1978, 1986) while Fields et al. (1996) reported that xanthine oxidase generates free radical during its physiological activity. Therefore, it is possible that oxyradical generation occurs in *Achatina marginatus* when exposed to elevated levels of contaminants. Reactive oxygen species initiate lipid peroxidation, which is a consequence of oxidative stress (Halliwell & Cross 1994). Arnaud et al. (2000) reported that lipid peroxidation is a bioindicator of oxidative stress, which tallies with the result of this investigation.

Table 1. Levels of lipid peroxidation, ascorbic acid and xanthine oxidase activity in the snail *Achatina marginatus*. N = number of snails per sample. a = significantly larger values compared to the control group, b = significantly smaller values than the control group. Results are expressed as mean ± SE.

	Polluted site (n = 5)	Control site (n = 5)
Lipid peroxidation ($mmolcm^{-3}$)	106 ± 5.2^{a}	69 ± 3.3
Ascorbic acid (mgg^{-1} Fwt)	1.60 ± 0.08^{b}	2.80 ± 0.12
Xanthine oxidase activity (Unit S^{-1})	68 ± 3.4^{a}	42 ± 1.8

The fact the snails collected from the polluted site are under oxidative stress was further highlighted by the depletion of the non-enzyme antioxidant system. The level of ascorbic acid was significantly lower when the snails from the polluted environment were compared with those from the reference site (Table 1).

Xenobiotic-induced depletion of ascorbic acid levels had been published earlier (Sharma & Buettner 1993, Buettner & Jurkiewiez 1996). Ascorbic acid reacts with the peroxyl radicals before they reach the membrane (Khoja & Marzouki 1994), hence its absence exposes affected animals to the deleterious effects of reactive oxygen species causing oxidative damage. This may explain why it has been proposed that ingestion of vitamins protect animals from petroleum mediated oxidative cell damage (Achuba et al. 2005).

In summary, the increase in the activity of xanthine oxidase, as well as

in lipid peroxidation and reduced level of ascorbic acid could be a reflection of oxidative stress in snails from the polluted site. Therefore, the general response of *A. marginatus* to the environmental contaminants is useful bioindicator of environmental pollution and makes the animal a promising tool for environmental assessment.

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