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Short Research Communication

Catalase activity in liver of Kafue lechwe *(Kobus leche kafuensis)* as biomarker for oxidative stress in pollution studies of Kafue flats, Zambia

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Catalase activity in liver of Kafue lechwe inhabiting the Kafue flats was measured. Research has shown that the Kafue flats have been exposed to heavy metals and pesticides posing a potential destruction to the inhabitants of the area. Studies on catalase activity in fish from the Kafue river have shown the potential of catalase activity as a biomarker of oxidative damage. However it is unclear how these findings apply on land and semi-aquatic Kafue lechwe within the flats. This study investigated the catalase activity in livers of Kafue lechwe in two game management areas (GMAs) within the flats. The mean catalase activity for Blue Lagoon and Lochnivar GMAs was 6.60 and 7.60 (K) respectively. Lochnivar showed a slightly higher mean level of activity although not significantly different from Blue lagoon (P = 0.36). The Pearson's correlation, showed no association between age and activity (P = 0.37). There were no differences in activity between the sexes (P = 0.17). The activity levels were comparable to those determined in fish from the Kafue river suggesting that the Kafue lechwe could be exposed to similar pollutants in the environment and that catalase activity in Kafue lechwe could be a potential biomarker.

Key words: Catalase activity, kafue flats, kafue lechwe, oxidative stress, pollution

INTRODUCTION

Catalase is present in the peroxisomes of mammalian cells where the enzyme reduces insult to the body by breaking down toxic hydrogen peroxide (Slaninova et al., 2009). In general, insults to the body are either intrinsic, as products of biochemical processes within the body or extrinsic arising from organic and inorganic environmental pollutants that induce oxidative stress by generating reactive oxygen species (ROS). Environmental pollutants especially heavy metals promote oxidative damage by directly increasing the cellular concentration of ROS and by reducing the cellular antioxidant capacity (Pinto et al., 2003). The Kafue flats has had its share of environmental pollutants in the form of heavy metals such as zinc, copper, lead and cadmium arising from the Copperbelt mining area (Norrgren et al., 2000). Other studies have also found pesticides and herbicides in water and fish in the area (Syakalima et al., 2006).

Different strategies for assessing pollution or contamination of the environment are currently being explored and they include direct measurement of pollutants in the environment (Sichilongo and Torto, 2006) or indirectly assessing biomarkers in bio-indicator organisms (Kendall et al., 2001). Among the biomarkers of oxidative stress that have gained considerable interest in the field of eco-toxicology, is the antioxidant enzyme catalase. A number of studies on the responses of catalase activity have been observed in animals exposed to organic or metallic contaminants in both field and laboratory experiments. Catalase has been shown to be either induced or sometimes inhibited by environmental pollutants depending on the dose, the species or the route of exposure (Romeo et al., 2000; Sanchez et al., 2005)

Only one study along the Kafue river has demonstrated changes in catalase activity in fish relative to different levels of pollution along the river (Syakalima et al., 2006). This

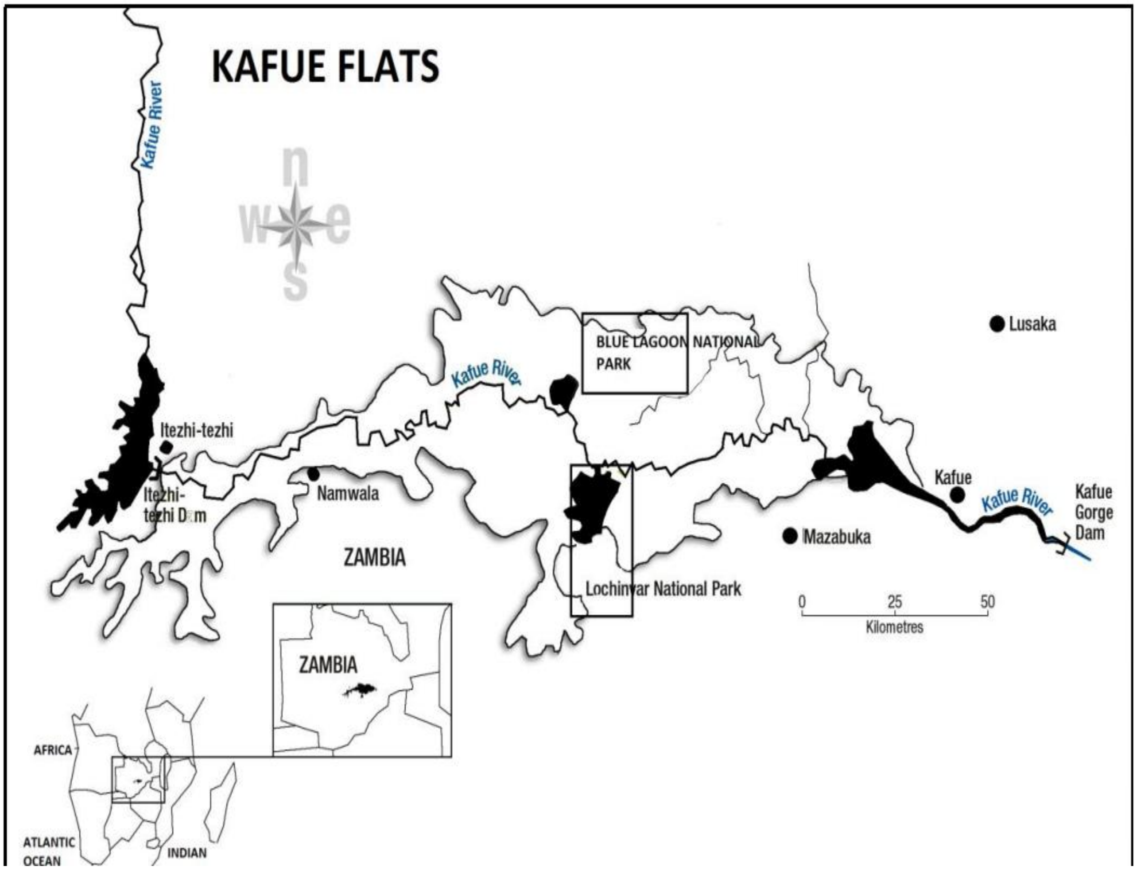

Figure 1: Map showing the Kafue flats and the location of Blue lagoon and Lochnivar national parks of Zambia (Adapted from http://wwf.panda.org)

study has shown the potential of catalase as a useful and sensitive marker of pollution in the area. However, other than in fish, this enzyme has not been assessed in other bio-indicator organisms in the area especially those that are semi-aquatic such as the Kafue lechwe (*Kobus leche kafuensis*).

The Kafue lechwe is a semi-aquatic antelope of the Kafue flats of Zambia and the most abundant animal species in the Blue lagoon and Lochnivar national parks. The Kafue river serves as a source of water for lechwe and other animals found in the two national parks. Various reports indicate that the river is substantially polluted because of discharges from the mines, industries and agricultural activities. These findings have raised concern on the health effects of the wildlife dependent on the river. Previous studies in the Kafue lechwe have shown evidence of the presence of heavy metal accumulation in the liver and its impact has been assessed by determining metallothionein (MT1) mRNA expression levels (M'kandawire et al., 2012, Syakalima et al., 2006).

Another study has also shown that the Kafue lechwe are exposed to endocrine disruptors (Sichilongo and Torto, 2006). In addition, responses to cytochrome P450 as potential biomarkers of persistant organic pollutants have also been determined in the Kafue lechwe (M'Kandawire et al., 2014). The use of biomarkers in the assessment of ecosystems' health allows for detection of subtle biological changes in the host. This provides an information base for biomarkers of pollution for specific hosts such as the lechwe necessary for research and environmental risk assessment. Therefore, this research aims to investigate the catalase activity in lechwe. The data generated may provide a useful database for future investigations of pollutant effects on antioxidant systems in lechwe.

MATERIALS AND METHODS

Sampling sites and samples

Twenty Kafue lechwe liver samples were collected in 2009 from Lochnivar (n=10) and Blue lagoon game management areas (GMAs) (n=10) respectively. The sites of collection are shown in Figure 1. The Zambia Wildlife Authority (ZAWA) issued the permit to obtain samples from lechwe for disease surveillance. Postmortems of the lechwe were carried out, and liver samples were collected using forceps and scissors and placed in labelled polythene bags, then wrapped in labelled aluminum foil and preserved in liquid nitrogen. The

Table 1 .List of samples from Kafue lechwe (BL; Blue Lagoon, L; Lochnivar) used in the study

No.	Sample	Age (years)	Sex
1	BL1	3	Male
2	BL2	5	Female
3	BL3	7	Male
4	BL4	3.5	Male
5	BL5	8.5	Male
6	BL6	8.5	Male
7	BL7	6.5	Male
8	BL8	5.5	Male
9	BL9	3.5	Male
10	BL10	12	Female
11	L1	18	Male
12	L2	14	Male
13	L3	6.5	Male
14	L4	8.5	Female
15	L5	12	Female
16	L6	12	Female
17	L7	12	Female
18	L8	10.5	Male
19	L9	11	Male
20	L10	6	Male

samples were then stored at -80˚C in the laboratory after transportation from the field. Information on the samples is indicated in Table 1. The age was determined by formation of rings on horns in males and wear and tear of teeth in both males and females.

Measurement of catalase activity

Catalase activity (Mueller et al., 1997) was measured in liver samples that had been preserved at -80°C, as described in literature (Cohen, et al., 1970) with some modifications (Syakalima et al., 2006). Briefly, 0.5 g of liver sample was weighed and homogenised in 1ml of 0.01 M ice-cold phosphate buffer, pH 7.0. The homogenate was centrifuged at 10,000 rpm for 10min at 4°C and supernatant was collected.

Protein concentration was determined by the method of using the Folin's reagent and bovine serum albumin as a standard (Lowry et al., 1951). The measurement of catalase activity was carried out as previously described (Syakalima et al., 2006). The absorbance was read on a UV-Vis spectrophotometer (Hitachi UV-VIS Single beam spectrophotometer Model 100-20) at 472 nm within 30-60 sec from the addition of $KMnO_4$. Under the conditions described above, the decomposition of H_2O_2 by catalase follows first-order kinetics as given by the equation : k=log (S_0/S_3) x 2.3/t, Where; k is the first-order reaction rate constant and is being used as a measure of catalase activity, t is the time interval (3 min) over which the reaction was measured, S_0 is the substrate concentration at time zero (standard absorbance-blank absorbance), S_3 is substrate concentration at 3 min (standard absorbance-sample absorbance).

Data analysis

Descriptive statistical analyses and graphs were performed using R statistical software (R Core Team, 2014, Vienna Austria). The significance of results was performed at $p<0.05$.

RESULTS

Catalase activity was measured in livers of lechwe from Blue lagoon (n=10) and Lochnivar (n=10) GMA. The mean catalase activity for Blue lagoon and Lochnivar national parks were 6.60 and 7.60 K, respectively. Although Lochnivar showed a slightly higher mean level of activity than Blue lagoon (Figure 2), there was no significant difference between the two regions (Wilcoxon rank sum test: W=37.5, N=10, P=0.36). There was no association between age and catalase activity. (Spearman's rank correlation: S = 0.211, N = 5, P = 0.37). In addition, females showed a slightly elevated mean catalase activity (Figure 3) although there was no difference in catalase activity between the sexes within the GMAs (Wilcoxon rank sum test: W=59, N = 10, P= 0.17).

DISCUSSION

The effect of pollution on domesticated and wild animals is of significance in Zambia because of the extensive mining and agricultural activities in the country. The situation is aggravated by indiscriminate disposal of chemical waste materials from mining and other chemical industries into

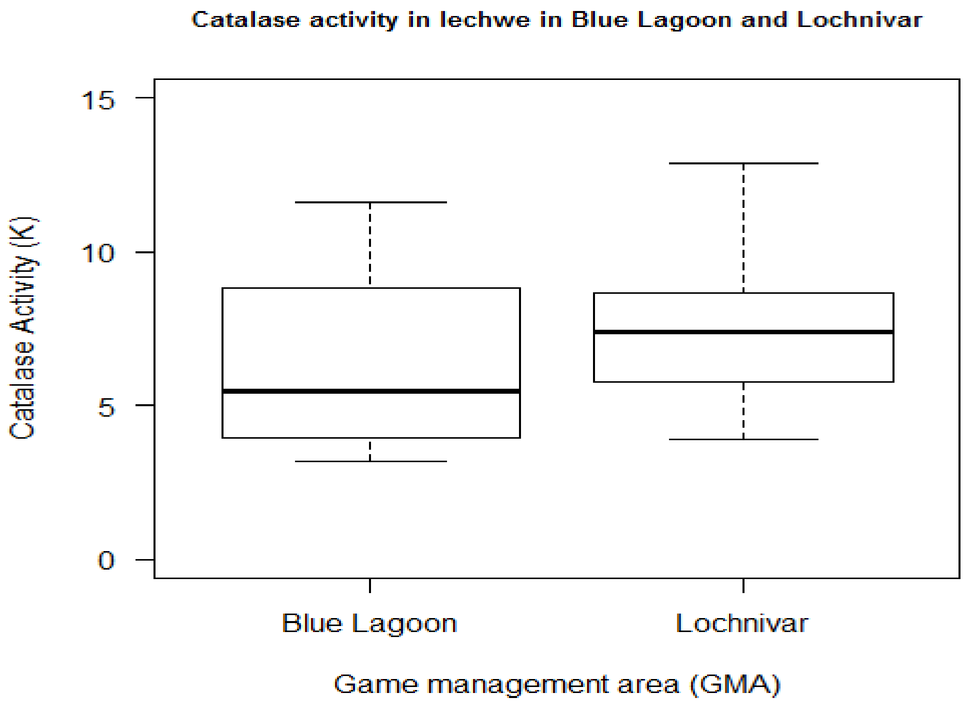

Figure 2: Box plot showing catalase activity in Kafue lechwe from Blue lagoon (*n*=10) and Lochnivar (*n*=10) GMAs

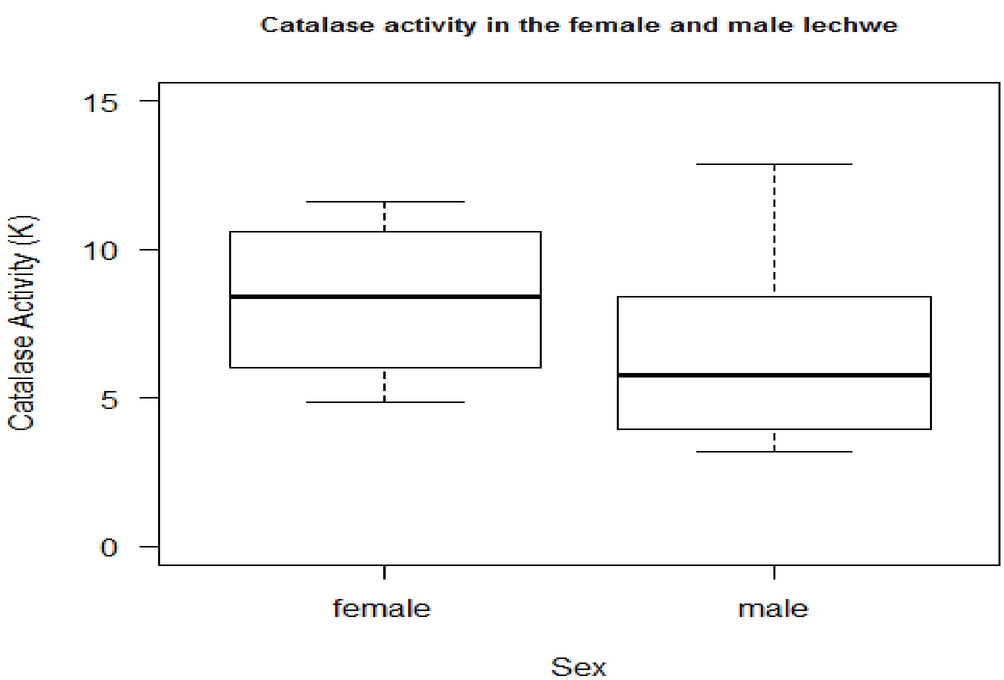

Figure 3: Box plot showing catalase activity in female (*n*=6) and male (*n*=14) Kafue lechwe in the Blue lagoon and Lochnivar GMAs

the rivers (Gilman, 1997; Marais, 1981; Norrgren et al., 2000). The chemical constituents discharged into the water bodies are important because of the potential negative impact they have on the health of humans and other higher animals (Iossifidou et al., 1999; Kamarianos et al., 1997). A number of studies have detected a wide range of potential contaminants in the Kafue river and its ecosystem (Bäckström and Jonsson, 1996; Pettersson et al., 2000). In addition, there is evidence suggesting that the levels of contamination are not evenly distributed in the Kafue river basin (ECZ, 2000; Kambole and Chilumbu, 2001). Studies carried out in lechwe inhabiting the Kafue flats have clearly demonstrated that the Kafue lechwe is exposed to heavy metals and endocrine disrupters (M'kandawire et al., 2012; Sichilongo and Torto, 2006; Syakalima et al., 2001). In this study we sought to investigate the biological effects of the pollutants on lechwe in the Blue lagoon and Lochnivar GMAs by analyzing catalase activity in the liver.

This study has shown that catalase activity has potential application as a biomarker for oxidative stress in the lechwe and other wildlife, and is comparable to what was observed in fish collected along the Kafue river (Syakalima et al., 2006). Although the assessment of activity in fish in the area was able to reveal a gradient along the Kafue river, our study comparing Blue lagoon and Lochnivar GMAs failed to detect any differences. This could suggest that the levels of contamination in the two regions are similar or that the substances responsible for eliciting the effect on the lechwe were comparable.

The absence of reference values for catalase activity in lechwe precluded the direct comparison with regard to how low or high the values were. Nevertheless, a crude comparison of the values in lechwe with those obtained from fish livers (Syakalima et al., 2006) showed that the activity in lechwe were significantly higher than those of fish obtained from Chingola in Zambia but not those from Kafue river basin. These findings would be appropriate especially for fish from Kafue river basin, as the exposure of fish to catalase-inducing agents would be expected to be reasonably higher than in the lechwe as fish are continuously exposed. The protocols currently available for measuring the effects of environmental contamination are diverse and so are the inferences derived there from. Although direct detection methods for pesticides or metals in the soil, vegetation and animal organs are widely used, assessment of *in vivo* responses such as catalase activity has its advantages. Such responses provide a direct link to the damage caused by inducing agent in the bio-indicator organism under evaluation.

Despite our investigation showing that the level of catalase activity in lechwe in Blue lagoon and Lochnivar GMAs were similar, previous studies found that differences occurred in Cytochrome p450 1A1 (CYP1A1), Cytochrome p450 3A (CYP3A) and Metallothionein 1 (MT1) mRNA expression levels between the two GMAs (M'Kandawire et al., 2012; M'kandawire et al., 2014). The mRNA expression level of CYP1A1 was significantly higher in Lochnivar GMA than Blue lagoon GMA. However, mRNA expression levels of CYP3A and MT1 was higher in Blue lagoon GMA than Lochnivar GMA. Assessment of the activity in the two areas failed to corroborate these findings probably because catalase may not be as sensitive as the mRNA expression method. Another plausible explanation for the lack of difference could be that the inducing agents may have the same effect on the micro environment inducing catalase thus negating the differences in the precipitating agents (Bussolaro et al., 2010; Holen and Olsvik, 2014). Nevertheless, in the early stages of an investigation when data is still scanty or absent, a battery or complementary tests are necessary to enable validation of the parameters under assessment or evaluation

Sex of the Lechwe did not influence catalase activity despite females showing an elevated mean level of activity than the males probably due to the smaller number of females that were available. It was also noted that the age of the animals was not a significant factor on the catalase activity, a finding attributable to the block nature of the age range (3-12 years) of study units. This could suggest that once animals have been on the grazing pastures for a minimum duration of time the levels of catalase activities remained above a baseline. Determining the catalase activity in a wider age range would probably provide more information on the effect of age.

As earlier mentioned, there are few studies that have looked specifically at catalase activities in lechwe beside those in fish (Ferreira et al., 2005; Sayeed et al., 2003; Syakalima et al., 2006). Catalase has been shown to be sensitive to constant and incremental exposure to contaminants and therefore a potentially useful marker (Richardson et al., 2008). Our study therefore provides useful preliminary information on the usefulness of catalase in pollution studies involving lechwe. Sample size is usually a big challenge with all studies involving wildlife because of national laws on conservation. However it is likely that a larger sample size would have been better and it would have given this study a much better correlation.

In conclusion catalase activity was successfully measured in the liver of lechwe from two GMAs in the Kafue flats. The determination of catalase activity in the lechwe is an easier, valuable and possible complementary test in the evaluation of environmental pollution in the Kafue river basin.

Conflict of interest statement

The authors of this paper declare that none of them have any financial or personal relationships with individuals or organizations that would unacceptably bias the content of this paper. The manuscript does not contain clinical or patient data.

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