

Serum Biochemistry and Enzymatic Biomarker of Gercacinid Crabs from Mangrove Swamp of the Lagos Lagoon, Nigeria

Rasheed O. Moruf^{1*}, Musa I. Abubakar² and Fatima B. Abubakar¹

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¹Department of Fisheries and Aquaculture, Bayero University, Kano, Kano State, Nigeria

²Department of Aquaculture and Fisheries, University of Ilorin, Kwara State, Nigeria

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ABSTRACT

Environmental factor is known to influence crab immune chemistry. This study presents the haemato-biochemical parameters and enzymatic biomarker of gercacinid crabs (*Cardisoma guanhumi* and *Cardisoma armatum*) from mangrove swamp of the Lagos Lagoon using standard procedure. The haemocyte subpopulations were higher in *C. guanhumi* than the percentage obtained in *C. armatum* with no significant difference ($P > 0.05$). Significantly, higher range values of aspartate aminotransferase ($12.03 \pm 0.35 \mu\text{i}^{-1}$), alanine aminotransferase ($10.07 \pm 0.58 \mu\text{i}^{-1}$), alkaline phosphatase ($89.94 \pm 3.38 \mu\text{i}^{-1}$), lactate dehydrogenase ($124.86 \pm 0.70 \mu\text{i}^{-1}$) and urea ($33.90 \pm 2.00 \text{mg dL}^{-1}$) were recorded in *C. guanhumi*. Examined samples of gercacinid crabs showed significant difference only in malondialdehyde (among the enzymatic biomarkers) with mean values of $6.32 \pm 0.05 \text{Hmol/mg pro}$ and $9.36 \pm 0.27 \text{Hmol/mg pro}$ for *C. guanhumi* and *C. armatum* respectively. The haemato-biochemical parameters of the gercacinid from the studied location varied across species.

Keywords: Biomarker; crab; haematology; serum biochemistry; Lagos Lagoon

Introduction

The Lagos Lagoon which serves as a major source of economically important fish, crustaceans and molluscs for the inhabitants of Lagos and its neighbouring communities is under pressure from pollutants like untreated sewage, sawdust, petrochemical materials, detergent and industrial effluents emanating from surrounding city centres. Most water bodies in Nigeria, especially Lagos serve as a sink for the disposal of waste from about 2000 medium and large scale industries located in the metropolis in an unregulated manner (Osuala *et al.*, 2020). An important ecological ramification of increasing population pressure, poor sewage

system, industrialization and poor waste management in Nigerian's coastal area is that pollutants freely find their way unabated into our coastal waters through drains, canals, rivers, creeks and lagoons that act as conduits (Balogun and Ajani, 2021). The release of organic and chemical pollutants into the aquatic environment results in some changes, which may threaten functional attributes, integrity and existence of aquatic organisms (Emeka *et al.*, 2020). Evidence suggested that much of this exposure to contaminants are presented as multiple mixtures of chemicals, the toxic effect of which are unknown, particularly over longer time scales (Osuala *et al.*, 2020). There have been numerous investigations into the ecology and

Corresponding Author: Rasheed O. Moruf

Department of Fisheries and Aquaculture, Bayero University, Kano, Kano State, Nigeria

Email: tunjimoruf@gmail.com Phone: +2348022429983



health of the Lagos Lagoon and the linear assessment of historical record points to increasing stress (Benson *et al.*, 2020; Moruf *et al.*, 2022a; Ayodele *et al.*, 2023).

Page | 4540 Haematological measures have recently emerged as viable indicators for assessing the impact of chemical pollutants on crustaceans. The reactions of haematological parameters to chemical stimuli are typically non-specific. Nonetheless, they may be useful in evaluation studies since they give an indicator of the general physiology and health status of the organism being studied (Koelmans *et al.*, 2016). The effect of pollutants on crustaceans can also be assessed at different levels of biological organisation using biomarkers. All biological systems generate endogenous reactive oxygen species (ROS) during their aerobic metabolism and energy production (ATP generation) in the mitochondria (Nwabueze *et al.*, 2020). Some pollutants have the potential to produce ROS in organisms which are exposed to them, thereby causing an increase in the level of ROS in the body of the organisms (Birben *et al.*, 2012).

An organism's defence systems of enzymatic and non-enzymatic anti-oxidants help to reduce the presence of ROS within the organisms. Enzymatic antioxidant defences of living systems include: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GRx) and glutathione S-transferase (GST). An increase in the activity of these enzymes is an indication (biomarker) of the effect of pollutants that generate ROS (Nwabueze *et al.*, 2020). Lipid peroxidation is also a biomarker of oxidative damage in organisms inhabiting polluted ecosystems. It is generated by free radicals in an organism. An increase in free radicals causes over production of malondialdehyde (MDA) which is one of the final products of polyunsaturated fatty acids peroxidation in the cells of organisms (Nwabueze *et al.*, 2020).

The toxicity, tissue distribution, oxidative stress marker and haematological alterations of

contaminants in crabs have been studied by a number of researchers (Jesuniyi *et al.* 2020; Ugwu *et al.* 2021; Moruf *et al.* 2022b). Because crabs are so closely linked to the mangrove ecosystem, the serum will detect measurable physiological changes in the crustacean far faster than any other physiological evaluation measure (Gomes *et al.*, 2017). Haematology and clinical chemistry analysis can provide substantial diagnostic information once reference values are established. Unfortunately, the number of studies in which reference intervals have been determined for crabs is limited in Nigeria. The aim of this research is to evaluate the baseline serum profile and enzymatic biomarkers of gecaciniid species from mangrove swamp of the Lagos Lagoon in Nigeria.

Materials and Methods

Sample collection

The Lagos Lagoon is one of the meandering networks of lagoons and creeks found along the coastline of Southern Nigeria. The study site (Latitude 6°26'- 6°37'N and Longitude 3°23'-4°20'E) serves as waste disposal point for the most communities in Lagos, one of the most highly populated coastal cities in Nigeria (Moruf *et al.*, 2018). Sources of pollution into the lagoon include effluents from brewery, industries, solid wastes from slaughter houses, sawmills as well as domestic and untreated sewage. Samples of *Cardisoma spp* were collected inside and along the mangrove area by hand picking in hand gloves, identified (according to taxonomic keys of Schneider, 1990) and then transported to the laboratory, acclimatized for 48hrs and fed with sampling location mud rich organic matter until further analysis.

Laboratory analysis

Crab haemolymph was drawn with a 23G syringe from the juncture between the bases of the ischium of the fifth walking leg. The haemolymph was collected into a syringe flushed with 1mL of anticoagulant (0.3 M NaCl, 0.1 M glucose, 30 mM

Sodium citrate and 26 mM Citric acid), transferred into a 5mL lithium heparin bottle kept in an ice chest and haemolymph of crabs were analyzed immediately for haemocyte morphology, haematological and biochemical indices using an improved Neubauer haemocytometer (Blaxhall and Daisley, 1973). Individual crab haemolymph aliquots were transferred to the haemocytometer and manually counted. The morphotypes of haemocytes were determined, and 100 cells from each slide were counted. To achieve an absolute count, the percentage of each counted cell type was computed and multiplied by the overall haemocyte population count.

The serum was assayed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), phosphatase alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and urea according to standard methods (Coles, 1986). Excised muscle tissues of the crabs stored at $-20\text{ }^{\circ}\text{C}$ were later thawed and homogenized for the assays of superoxide dismutase (SOD), catalase (CAT), glutathione transferase (GST), reduced glutathione (GSH) and glutathione peroxidase (GPx) and malondialdehyde (MDA) (Lushchak *et al.* 2005; Bertholdo-Vargas *et al.* 2009). Protein was determined spectrophotometrically using the Bio-Rad DC protein assay kit (Richmond, CA, USA) with bovine serum albumin as a standard (Lowry *et al.*, 1951).

Data analysis

Data obtained from the study were subjected to Microsoft Excel 2010 for descriptive statistics and student-t-test for identification of significant variation. Differences in means were considered significant when $P < 0.05$.

Results and Discussion

Haematological profile

Haematological parameters are closely related to the response of the animal to its environment, indicating that where the crab lives could exert some influence on the serum parameters. The haematology result of

the gercacinid crabs from the mangrove swamp of Lagos Lagoon obtained during the study period is shown in Figure 1. Total Haemocyte Count (THC) were $3850 \pm 0.05\text{ mL}$ and $3421 \pm 0.01\text{ mL}$ for *C. guanhumi* and *C. armatum* respectively. The haemocyte subpopulations were higher in *C. guanhumi* than the percentage obtained in *C. armatum* with no significant difference ($P > 0.05$). With respect to the haematological value of *C. armatum* from Abule-Eledu Creek (Lawal-Are *et al.*, 2021), it is apparent that a marked deviation of haematological indices for this study is evident as comparison of reference interval should be done with caution in respect to variation in environmental condition. Serum sampling, laboratory techniques, seasonal variation, size, genetic properties, sex, population density, lack of food supply, environmental stress and transportation could affect haematological data (Wachap *et al.*, 2019). This is in resonance with the position of Sanni *et al.* (2020), who stated that if environmental factors or stressors are severe and long lasting, the response then becomes mal-adaptive and threatens the health of the organisms and its wellbeing.

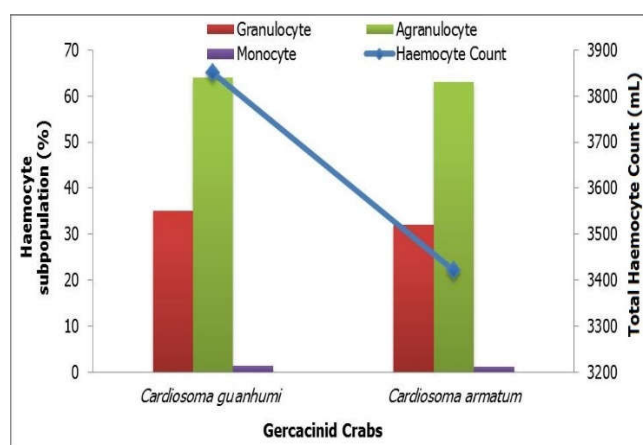


Fig. 1. Haematological indices of gercacinid crabs from the mangrove swamp of Lagos Lagoon

Serum biochemical profile

The result of the baseline serum biochemical profile of gercacinid crabs from the mangrove swamp of Lagos Lagoon is presented in Table 1. Significantly ($P < 0.05$), higher range values of AST ($12.03 \pm 0.35 \mu\text{I}^{-1}$), ALT ($10.07 \pm 0.58 \mu\text{I}^{-1}$), ALP ($89.94 \pm 3.38 \mu\text{I}^{-1}$), LDH ($124.86 \pm 0.70 \mu\text{I}^{-1}$) and urea ($33.90 \pm 2.00 \text{mg.dL}^{-1}$) were recorded in *C. guanhumi*. The increase may be a direct consequence of stress induced protein metabolism in the tissue of the crab. ALT and AST are used as general indicators of liver function, with ALT being more liver-specific (Wu *et al.*, 2014). Changes in AST could be attributed to interference in the immune system of the crab, resulting to cell damage or a way in which the crabs are reacting to the exposure of contaminant (Sanni *et al.*, 2020). This finding is in agreement with earlier investigators who have exposed these crabs to different chemicals and contaminant (Jesuniyi *et al.*, 2020; Ugwu *et al.*, 2021; Lawal-Are *et al.*, 2022). Variations in serum parameters have been attributed to responses to a changed physiological and energetic requirement and may be an early warning measure of stress before population declines are observed (Okunade *et al.*, 2020).

Enzymatic Biomarker

Antioxidant defense enzymes analyzed in the present study are generally involved in cellular protection against peroxidation damage and maintained redox equilibrium within the cell. The result of the enzymatic biomarker of gercacinid crabs from the mangrove swamp of Lagos Lagoon is shown in Table 2. Examined samples of gercacinid crabs showed significant difference ($P < 0.05$) only in Malondialdehyde (MDA) with mean values of $6.32 \pm 0.05 \text{Hmol/mg pro}$ and $9.36 \pm 0.27 \text{Hmol/mg pro}$ for *C. guanhumi* and *C. armatum* respectively. Relatively, higher range values of PRO ($21.03 \pm 0.66 \text{g L}^{-1}$), SOD ($110.76 \pm 21.38 \text{min/mg/pro}$), CAT ($4.69 \pm 0.93 \text{min/mg/pro}$), GST ($13.89 \pm 1.53 \text{Hmol/mg pro}$), GSH ($2.37 \pm 0.73 \text{Hmol/mg pro}$), GPx (48.25 ± 3.09

Hmol/mg pro) and MDA ($9.36 \pm 0.27 \text{Hmol/mg pro}$) were recorded in *C. guanhumi*.

Increase in antioxidant enzymes activities have been attributed to adaptive responses of the organism to counteract the oxidative effect of generated ROS or resistance to water pollutants toxicity against the damage caused by the excessive amount of oxygen free radicals and oxidative stress (Carvalho *et al.*, 2012). SOD-CAT system is the primary defense mechanism against such oxidative stress. The recorded high and relatively low levels of SOD activity observed in the crabs suggest potential stress from their locations. The apparent increase in glutathione levels in the organs suggests an adaptive and protective role of this biomolecule against oxidative stress induced by the anthropogenic activities. Furthermore, an increased GSH is often indicative of initiation of cellular defense mechanism in response to increasing concentration of free radical in the cell while a decrease may indicate an overrun of the antioxidant defense (Moruf, 2021).

MDA is a biomarker of oxidative damage that reflects the state of lipid peroxidation of the membrane of many living organisms. The result of lipid peroxidation in muscle tissues of the gercacinid crabs expressed by the values of MDA indicates the oxidative damage in a biological system. Oxidative damage set in when there is no equilibrium between the reactive oxygen species (ROS) generated as a result of bioaccumulation of contaminant and the antioxidant biomarker response (Lawal-Are *et al.*, 2019). Alternatively, the ROS overwhelm the production of antioxidant biomarkers. Protein modifications can also take place in the cytoplasm, either as a result of the action of regulatory enzymes or ROS. Oxidation of proteins is of importance in regulating protein function within the cell. Many proteins undergo regulatory steps altering their oxidative status prior to release from the endoplasmic reticulum or Golgi apparatus (Hetz *et al.*, 2020). The elevated lipid peroxidation concentration observed in *C. armatum*

is due to pollutants exposure which might be due to the microsomal metabolism of xenobiotic and microsome mediated redox cycling which gives rise to radicals capable of oxidizing membrane lipids.

The result corroborated the higher lipid peroxidation observed in domestic waste dumps exposed mangrove crab, *Sesarma huzardii* (Uuse et al., 2018).

Table 1: Serum biochemical indices of gercacinid crabs from the mangrove swamp of Lagos Lagoon

Parameter	<i>Cardisoma guanhumi</i>			<i>Cardisoma armatum</i>			P-value
	Minimum	Maximum	Mean±SE	Minimum	Maximum	Mean±SE	
AST (μI^{-1})	11.50	12.56	12.03±0.35	1.01	3.31	1.81±0.75	0.00
ALT (μI^{-1})	9.20	10.93	10.07±0.58	4.89	8.89	6.39±1.26	0.05
ALP(μI^{-1})	84.20	95.68	89.94±3.38	63.13	68.30	64.86±1.72	0.00
LDH(μI^{-1})	123.81	125.90	124.86±0.70	86.97	91.47	88.51±1.48	0.00
Urea (mg.dL^{-1})	30.89	36.90	33.90±2.00	19.90	25.70	22.10±1.81	0.01

Keys: AST- Aspartate Aminotransferase, ALT- Alanine Aminotransferase, ALP - Alkaline phosphatase, LDH- Lactate dehydrogenase.

Table 2: Enzymatic biomarkers of gercacinid crabs from the mangrove swamp of Lagos Lagoon

Parameter	<i>Cardisoma guanhumi</i>			<i>Cardisoma armatum</i>			P-value
	Minimum	Maximum	Mean±SE	Minimum	Maximum	Mean±SE	
PRO (g L^{-1})	12.35	34.16	20.84±3.63	19.90	22.20	21.03±0.66	0.97
SOD (min/mg/pro)	46.15	84.47	69.47±11.82	64.10	187.62	110.76±21.38	0.21
CAT (min/mg/pro)	2.01	4.66	3.67±0.84	1.86	7.741	4.69±0.93	0.49
GST(Hmol/mg pro)	8.82	14.35	11.64±1.60	9.89	18.4	13.89±1.53	0.38
GSH (Hmol/mg pro)	0.11	2.73	1.47±0.76	1.26	5.00	2.37±0.73	0.45
GPx (Hmol/mg pro)	42.15	45.55	43.83±0.98	42.51	59.30	48.25±3.09	0.33
MDA (Hmol/mg pro)	6.23	6.39	6.32±0.05	8.49	10.10	9.36±0.27	0.00*

Keys: PRO- Protein, SOD- Superoxide dismutase, CAT- Catalase, GST- Glutathione transferase, GSH- Glutathione, GPx: Glutathione Peroxidase, MDA- Malondialdehyde. *-Significant difference ($P < 0.05$).

The study provides reference values for haematology, serum biochemical and enzymatic biomarkers of gercacinid crabs, useful when performing health assessments on these species. No

remarkable difference was observed in preliminary haematology values obtained for *Cardisoma guanhumi* and *Cardisoma armatum*. Regarding the preliminary serum biochemistry, significant ($P <$

0.05) higher range values of AST, ALT, ALP, LDH and urea were recorded in *C. guanhumi*. The increase may be a direct consequence of stress induced protein metabolism in the tissue of the crab. The results of the present study provide useful information for monitoring changes in the health status of crab population.

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