SERUM BIOCHEMISTRY AND ENZYMATIC BIOMAKER OF GERCACINID CRABS FROM THE MANGROVE SWAMP OF LAGOS LAGOON

Abstract

Serum biochemistry and antioxidant enzyme activity can provide substantial diagnostic information once reference values are established. This study presents the haemato-biochemical parameters and enzymatic biomarker of gercacinid crabs from the mangrove swamp of Lagos Lagoon using standard procedure. Total haemocyte counts were 3850±0.05 mL and 3421±0.01 mL for *Cardisoma guanhumi* and *Cardisoma armatum* respectively. 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±0.35 μi^{-1}), alanine aminotransferase (10.07±0.58 μi^{-1}), alkaline phosphatase (89.94±3.38 μi^{-1}), lactate dehydrogenase (124.86±0.70 μi^{-1}) and urea (33.90±2.00 $mg.dL^{-1}$) were recorded in *C. guanhumi*. Examined samples of gercacinid crabs showed significant difference only in malondialdehyde with mean values of 6.32±0.05 Hmol/mg pro and 9.36±0.27 Hmol/mg pro for *C. guhanhumi* and *C. armatum* respectively. Relatively, higher range values of protein (21.03±0.66 g L^{-1}), superoxide dismutase (110.76±21.38 min/mg/pro), catalase (4.69±0.93 min/mg/pro), glutathione transferase (13.89±1.53 Hmol/mg pro), glutathione (2.37±0.73 Hmol/mg pro), glutathione peroxidase (48.25±3.09 Hmol/mg pro) and malondialdehyde (9.36±0.27 Hmol/mg pro) were recorded in *C. guhanhumi*. The results of the present study provide useful information for monitoring changes in the health status of crab population.

Keywords: Biomarker, Haematology, serum biochemistry, crab, Lagos Lagoon

INTRODUCTION

The Lagos Lagoon which serves as a major source of fish and economically important crustaceans and molluscs for the inhabitants of Lagos is under pressure from pollutants such as untreated sewage, sawdust, petrochemical materials, detergent and industrial effluents. The release of organic and chemical pollutants into the aquatic environment results in some changes, which may threaten functional attributes, the integrity and existence of aquatic organisms (Emeka et al. 2020). Serum samples from test organisms can be obtained on a regular basis, providing for a non-destructive approach to effect assessment (Moruf and Lawal-Are, 2018). 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 toxicity, tissue distribution, oxidative stress marker and haematological alterations of contaminants in crabs have been studied by a number of researchers (Usese et al. 2018; Lawal-Are et al. 2019; Jesuniyi et al. 2020; Sanni et al. 2020; Lawal-Are et al. 2021; Ugwu et al. 2021). 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 fish is limited (Ayoola et al. 2014). The aim of this research is to evaluate the baseline serum profile and enzymatic biomarkers of gecacinid species (*Cardisoma guanhumi* and *Cardisoma armatum*) from the mangrove swamp of Lagos Lagoon.

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 (Moruf and Lawal-Are, 2015). The study site (Latitude $6^o26'$-

$6^o37'$N and Longitude $3^o23'$-$4^o20'$E) serves as waste disposal point for the most communities in Lagos, one of the most highly populated coastal cities in Nigeria. 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, transported to the laboratory, acclimatized for 48hrs and fed with sampling location mud rich organic matter until further analysis.

Laboratory Procedure

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 Neubaeur heamocytometer according to methods described by Blaxhall and Daisley (1973). The serum was assayed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), phosphatase alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and urea according to methods described by Coles (1986). Excised muscle tissues of the crabs stored at −20 °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) , following the protocol described by Lushchaks et al. (2005) and 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, based on the method of Lowry et al. (1951).

Data Analysis

Data obtained from the study were subjected to Microsoft Excel 2010 for descriptive statistics to establish means and standard deviations, one-way analysis of variance 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±0.05 mL and 3421±0.01 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 reported by Ugwu 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. According to Wachap et al. (2019), serum sampling, laboratory techniques, seasoned variation, size, genetic properties, sex, population density, lack of food supply, environmental stress and transportation could affect haematological data. 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.

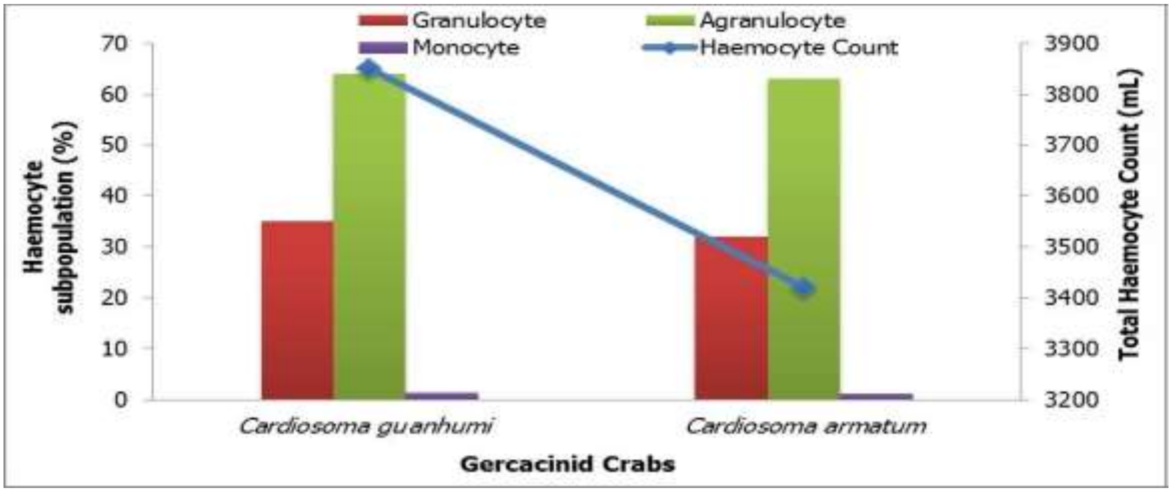

Figure 1: Haematological indices of gercacinid crabs from the mangrove swamp of Lagos Lagoon

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±0.35 μI^{-1}), ALT (10.07±0.58 μI^{-1}), ALP (89.94±3.38 μI^{-1}), LDH (124.86±0.70 μI^{-1}) and urea (33.90±2.00 $mg.dL^{-1}$) 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). According to Sanni et al. (2020), 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. This finding is in agreement with earlier investigators who have exposed these crabs to different chemicals and contaminant (Jesuniyi et al. 2020; Lawal-Are et al. 2021; Ugwu et al. 2021). 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 (Moruf and Lawal-Are, 2018; Okunade et al. 2020).

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

Parameter	*Cardisoma guanhumi*			*Cardisoma armatum*			*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.

Enzymatic Biomarker

The result of the enzymatic biomaker 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±0.05 Hmol/mg pro and 9.36±0.27 Hmol/mg pro for *C. guhanhumi* and *C. armatum* respectively. Relatively, higher range values of PRO (21.03±0.66 g L^{-1}), SOD (110.76±21.38 min/mg/pro), CAT (4.69±0.93 min/mg/pro), GST (13.89±1.53 Hmol/mg pro), GSH (2.37±0.73 Hmol/mg pro), GPx (48.25±3.09 Hmol/mg pro) and MDA (9.36±0.27 Hmol/mg pro) were recorded in *C. guhanhumi*. 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 (Lawal-Are et al. 2019). 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* (Usese et al. 2018). 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.

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

Parameter	*Cardisoma guanhumi*			*Cardisoma armatum*			*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).

CONCLUSION

This study provides reference values for some haematology, serum biochemical and enzymatic biomarkers of gercacinid crabs, which might be useful when performing health assessments on this 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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