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Partial purification of invertase from *Momordica charantia* (bitter melon) by three phase partitioning (TPP) method

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The single step three-phase partitioning (TPP) method was evaluated for the purification of invertase from *Momordica charantia.* Optimum conditions for TPP method, that is, 70% ammonium sulfate saturation, 1:0.5 t-butanol ratio and 30 min incubation time lead to 20.28% efficiency and 10.48 fold partial purification. Total protein decreased from 34.86 mg to 0.68 mg in the homogenate, and the specific activity of the enzyme increased from 21.67 to 227.02 U/mg protein. The purified enzyme showed maximum activity in 0.3 M pH 5.0 buffer at 50°C. In conclusion, the optimized single step TPP method revealed better purification.

Key words: Three-phase partitioning (TPP), invertase, *Momordica charantia,* enzyme purification.

INTRODUCTİON

Invertase (β-fructofuranosidase, EC 3.2.1.26) catalyses the hydrolysis of sucrose into glucose and fructose. In general, *Saccharomyces cerevisiae* invertase is used in the food and beverage industries. Invertase is commonly used for the production of non-crystallizable (invert) sugar syrup from sucrose. Invert sugars have a wide range of uses from fermentation to the manufacture of artificial honey and plasticizing agents. (Guimaraes et al., 2009; Hussain et al., 2009; Kotwal and Shankar 2009; Akardere et al., 2010). Invertase has been purified using various conventional purification processes, such as salting out, gel filtration, ultrafiltration, ion-exchange and affinity chromatographies (Wiseman, 1995). However, most of these processes involve multi-steps that decrease enzyme yields. These multi-step processes also affects product efficiency in terms of application difficulties and increased costs (Wiseman, 1995).

A simple three-phase partitioning (TPP) method was developed by Tan and Lovrien (1972) for the separation and purification of target proteins, which is more economically efficient This technique comprises mixing the crude protein extract with certain proportions of ammonium sulfate and t-butanol to generate three phases. The upper t-butanol phase contains non-polar compounds; whereas, the lower aqueous phase contains polar compounds with the two phases seperated by an interfacial protein precipitate containing the target protein. It is generally believed that the separation process involves a combination of different operating principles including kosmotropy, salting out, co-solvent precipitation,

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isoionic precipitation, osmolytic electrostatic forces, conformation tightening, and protein hydration shifts, which all contribute to protein precipitation at the medium phase (Dennison and Lovrien, 1997; Lovrein et al., 1987). Proteins display various behaviors under these conditions, depending upon their source, molecular weight, hydrophobicity, charge, temperature and isoelectric point (*pI*) (Dhananjay and Mulimani, 2009; Pike and Dennison, 1989; Dennison and Lovrein, 1997). Since the method was first reported in 1972, more than 100 research studies have been published on the use of TPP separation technology for the extraction and purification of a variety of biomolecules including proteins, enzymes, enzyme inhibitors, edible oils or lipids, carbohydrates and small-molecule organic compounds from diverse natural sources (Yan et al., 2017). Presently, invertase has been purified from five plant sources by the TPP methodology (Lovrein et al., 1987; Dhananjay and Mulimani, 2008; Kat and Yılmazer, 2013).

The objective of this study was to optimize the TTP purification of invertase from *Momordica charantia* fruits. *M. charantia* (bitter melon or bitter gourd) is a flowering vine in the Cucurbitaceae family. It is a tropical plant that is widely cultivated in Asia, India, East Africa, and South America. Its immensely bitter fruits are commonly used as an edible and natural remedy for treating diabetes (Abascal and Yarnell, 2005) and a wide range of illnesses (Bakare et al., 2010). Bitter melon is known to play an important role in glucose metabolism (Shibib et al., 1993; Platel and Srinivasan, 1997). Invertase is an enzyme which plays a role in glucose metabolism also, so, we choosed the bitter melon as an invertase source.

The methods used to purify different enzymes from other sources may not be suitable for a novel enzyme or source, because enzymes can be affected by various factors (ammonium sulfate concentration, ratio of t-butanol, pH and incubation time) in the method. Therefore, the method must be optimized, hence in the case of enzyme purification with TPP, the method has been optimized in the literature (Akardere et al., 2010; Chaiwut et al., 2010; Dhananjay and Mulimani, 2008; Duman and Kaya, 2013; Gagaoua et al., 2014; Hussain et al., 2009; Liu et al., 2006, Sagu et al., 2015).

This study is new in terms of the source from which the invertase is purified and the optimization of the TPP method for invertase.

MATERIALS AND METHODS

Ammonium sulfate ($(NH_4)_2SO_4$), copper (II) sulphate pentahydrate, Folin and Ciocalteu's phenol reagent, phosphoric acid, trisodium citrate, sodium carbonate, dialysis tubing cellulose membrane, sodium bisulfate, potassium sodium tartrate, t-butanol, sodium hydroxide, sucrose, 3,5-dinitro salicylic acid, ethylene diamine tetra acetic acid (EDTA), sodium dodecyl sulfate (SDS), bovine serum albumin (BSA) and trichloroacetic acid (TCA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other analytical grade chemicals were obtained from Merck (Darmstadt, Germany). *M. charantia* fruits were harvested from the southern region of Adana in Turkey.

Preparation of crude extract from *M. charantia*

M. charantia was obtained from a farm in August 2016 and then frozen at -20ºC. Frozen fruits were added to acetate buffer (0.2 M pH:5.0) at a 1:9 (w/v) ratio and ground in a blender. The milled sample was filtered through Whatman paper No.1. Preparation of this crude extract was carried out at 4°C. This sample was referred to as “crude extract” and used for further three-phase partitioning experiments.

Three-phase partitioning (TPP) of invertase

The TPP method described by Roy and Gupta (2002) was modified for enzyme purification. Solid crystalline ammonium sulfate was added to the crude extracts until desired saturation percentage was achieved, t-butanol was added at different ratios after complete dissolution of the ammonium sulfate. The mixture was allowed to stand for different times at room temperature and then centrifuged at 4500 rpm for 10 min to make easy the separation of phases. The upper phase was removed carefully and interfacial precipitate was dissolved in acetate buffer (0.2 M, pH 5.0). The samples were dialyzed against acetate buffer (0.2 M, pH 5.0), then total protein content of the samples and total and specific activity of invertase were determined. The optimum conditions (ratio of ammonium sulfate, ratio of t-butanol and incubation time) which resulted into maximum recovery of invertase were used in the purification procedure. The purified enzyme was characterized in order to determine some biochemical properties of the enzyme.

Optimization of TPP method

Optimization of ammonium sulfate ratio for invertase partitioning

The ammonium sulphate precipitation of crude extract was carried out at ratios of 40, 50, 60 and 70% (w/v) saturation at 4°C. Optimum concentration of $(NH_4)_2SO_4$ were investigated by using the 1:1 (v/v) ratio of t-butanol. The interfacial precipitate was collected and dissolved in 0.2 M acetate buffer (pH 5.0) for determination of enzyme activity and protein content. The ammonium sulphate concentration providing the highest invertase acticity was chosen for further study.

Optimization of t-butanol ratio for invertase partitioning

The crude extract was saturated with the optimal $(NH_4)_2SO_4$ concentration. Then, t-butanol was added to the samples at ratios of 1:0.5, 1:1, 1:1.5, and 1:2 (v/v). The t-butanol ratio that gave the highest enzyme activity was chosen for further study.

Optimization of ıncubation time for invertase partitioning

Incubation times evaluated were 15, 30 and 60 min. The incubation time with the highest invertase activity was chosen for further study.

Determination of invertase activity

The enzymatic activity of invertase was determined through the initial rates of sucrose hydrolysis reaction by the 3,5-dinitrosalicilic acid (DNS) method (Miller 1959; Sumner 1921). The assay mixture consisted of 0.2 ml of 0.5 M sucrose, 0.6 mL of acetate buffer (0.2 M, pH 5.0) and 0.2 ml of a sample containing invertase, which was then incubated at 37ºC for 30 min. Next, 1 ml of DNS reagent was

added and samples were heated in a boiling water bath for 10 min. Then samples were cooled to room temperature and the amount of reducing sugar was analysed with UV-visible spectrophotometer at 546 nm wavelength. One unit of invertase activity was defined as the amount of enzyme that resulted in the released 1 µmol of glucose from sucrose per minute.

Determination of protein content

Protein amounts of samples were determined by Lowry method using bovine serum albumin (BSA) as a standard (Lowry et al., 1951).

Characterization of invertase from M. charantia

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

SDS-PAGE was performed following the procedure of Laemmli (1970) using 15% separating gel on a Peqlab gel system unit with Biorad power suppy (45 mA, 200 V). Gels were then silver stained (Oakley et al., 1980) to visualize proteins.

Optimum temperature for M. charantia invertase

Standard assay procedure was performed at 25, 37, 50 and 60ºC. The specific activities were expressed as the ratio of enzyme activity obtained at certain temperature to the maximum activity obtained at the given temperature range.

Optimum pH for M. charantia invertase

Using the standard assay procedure, 0.2 M of the buffer systems (sodium acetate; pHs 4.0, 5.0, 6.0 and sodium phosphate; pHs 7.0, 9.0) were tested at optimum temperature. The remaining activity was determined and described as the specific activity.

Optimum buffer concentration for M. charantia invertase

The standard assay was applied using buffer concentrations at 0.2, 0.3, 0.5 and 0.7 M at optimum temperature and pH. Specific activity of the enzyme was calculated.

RESULTS AND DİSCUSSİON

Three-phase partitioning of invertase from *M. charantia*

For the single-step purification of invertase from *M. charantia*, TPP method was optimized in this study. There are several factors that affected the recovery of the desired protein in this process. So, the method needs some optimization to obtain suitable phase system for efficient TPP. The starting protein concentration (containing 7.56 U/ml of invertase activity) was 0.35 mg/ml. Invertase showed the highest activity at the interfacial precipitate predominantly at this study. If pI of protein are below the pH values of the process , the protein will remain in the aqueous phase because the protein is loaded negatively, thanks to negatively charged amino acid residues on the surface. Conversely, when pI of the protein is above the pH of the TPP medium, protein is precipitated and enriched in middle phase (Dennison and Lovrein, 1997 and Wang et al., 2011) There are some studies in the literature that the enzyme is in the middle phase. For example alpha-galactosidase enzyme was purified from pepino using TPP method, in which enzyme concentrated in the interfacial phase (Sen et al., 2011). In another study for the purification of Invertase from *Vitis labrusca*, the enzyme enriched middle phase after the TPP procedure (Kat and Yilmazer, 2013). Specific activity, ativity fold and yield were presented in Table 1.

As seen from the results, this single step invertase partitioning method depends on the amounts of ammonium sulfate and t-butanol, and also incubation time. pH is also the most important factor for this procedure. In the literature catalytic activities of invertases were measured at pH 5.0 (Miller, 1959; Sumner,1921) and also most of the invertase purification studies especially which are used plants as source at around pH 5.0 (Fotopoulos, 2005), therefore, pH wasn't optimized in this study.

The results of ammonium sulfate optimization are given in Figure 1. The highest invertase activty (1.23 U/mL) was observed at 70% (w/v) ammonium sulfate saturation. Optimum ammonium sulphate saturation for invertase purification is variable based on the source of the enzyme such as *Aspergillus oryzae* 30% (w/v) (Dhananjay and Mulimani, 2008), tomato (*Lycopersicon esculentum*) 50% (w/v) (Özer et al., 2010), Baker's yeast (*S. cerevisiae*) 50% (w/v) (Akardere et al., 2010), *Aspergillus sojae* 80% (w/v) (Lincoln and More, 2018), *Saccharum officinarum L.* 100% (w/v) (Hussain et al., 2009).

t-Butanol is the most convenient co-solvent, owing to its size and furcate structure, and also does not cause denaturation (Dennison and Lovrein, 1997; Dhananjay and Mulimani, 2008). The best results with 2.70 U/mL activity were obtained with 1:0.5 (v/v) crude extract: t-butanol ratio as illustrated in Figure 2. As the amount of t-butanol increase, the activity of the enzyme decreased in this study. Higher amounts of t-butanol can cause protein denaturation (Yan et al., 2017; Chaiwut et al., 2010). Similar ratios were reported for other species with 1:1 (v/v) for *A. oryzae* (Dhananjay and Mulimani, (2008), *V. labrusca* (Kat and Yılmazer, 2013) and tomato (*L. esculentum*) (Özer et al., 2010), the ratio is 1:0.5 (v/v) for *Baker's yeast (S. cerevisiae)* (Akardere et al., 2010).

Maximum activity of invertase was seen in 30 min incubation time as presented Figure 3. Invertase activity decreased sharply after 60 min. It means that enzyme may be denatured at this time period at that conditions.

M. charantia invertase was partialy purified 10.48-fold with a recovery of 20.28% by the TPP procedure as presented in Table 1. These results are similar to other studies that used multi-stage purification of invertase. For instance, invertase from *Aspergillus sojae JU12*. was purified by size exclusion chromatography with 5.41 fold and 10.87% recovery (Lincoln and More, 2018).

Table 1. Activity of invertase from *Momordica charantia* purified by three-phase partitioning.

Step	Activity (U/mL)	Protein (mg/mL)	Total protein (mg)	Specific activity (U/mg protein)	Total activity (U)	Yield (%)	Purification fold
Crude extract	7.56	0.35	34.86	21.67	755.50	100	1
TPP-Interfacial precipiate	5.11	0.02	0.68	227.02	153.24	20.28	10.48

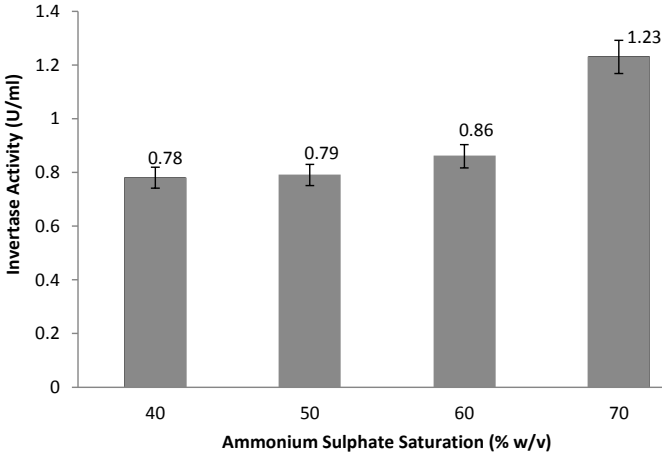

Figure 1. Effect of varying saturations of ammonium sulfate on purification of invertase by TPP. Ammonium sulfate was added to crude extract at ratios of 40, 50, 60 and 70% (w/v).

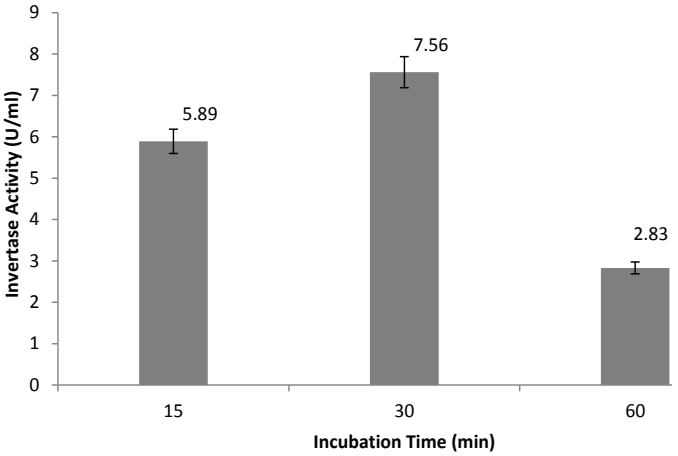

Figure 3. Optimization of incubation time for invertase partitioning. Ammonium sulphate and t-butanol were incubated in crude extract at 15, 30 and 60 min for the TPP.

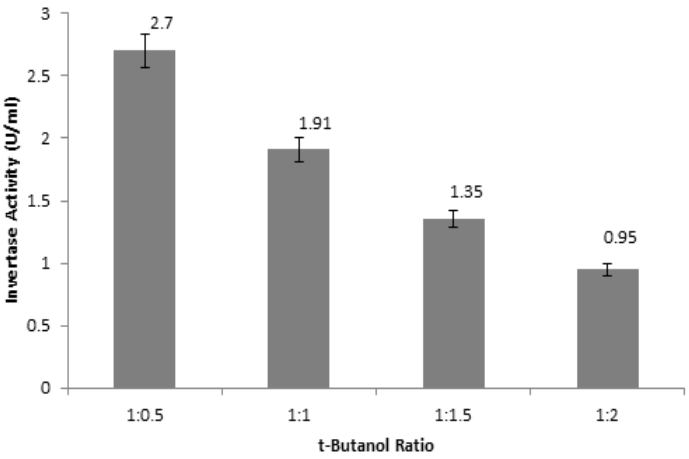

Figure 2. Optimization of crude extract to t-butanol ratio for invertase partitioning. t-butanol was added at ratios of 1:0.5, 1:1, 1:1.5, and 1:2 (v/v) to crude extract saturated with 70% ammonium sulfate.

Purification of invertase from bamboo cells achieved 64-fold purification with a recovery of 26% using interaction of ammonium sulfate precipitation, Dimethylaminoethyl (DEAE)-sephacel and gel filtration chromatographies (Liu et al., 2006). Invertase from sugarcane was purified by muli-stage; ammonium sulfate precipitation, anion exchange, hydrophobic interaction chromatography and gel filtration resulting in 13-fold purification with 35% recovery (Hussain et al., 2009). Guimaraes et al. (2009) used DEAE-cellulose and Sephacryl S-200 chromatographic methods resulting in 7.1-fold with a recovery of 24% for the purification of invertase from *Aspergillus ochraceus*.

The purification findings of current study (10.48-fold with a recovery of 20.28%) as seen in Table 1 are also better or close to some other TPP studies. For example AAO (aryl alcohol oxidase) from *Pleurotus ostreatus* was purified using TPP 10.19-fold with 10.95% activity recovery (Kumar et al., 2011). Dogan and Tari (2008) were also purified using exo-polygalacturonase from *Aspergillus sojae* to 6.7 purification fold with 25% activity recovery. PPO (polyphenol oxidase) from *Trachystemon orientalis L.* purified 3.59-fold with 68.75% activity recovery (Alici and Arabaci, 2016). Purification of inulinase from *Aspergillus niger* was achieved with 10.2 purification fold and 88% activity recovery (Kumar et al., 2011) which is enough to make TPP preferable. Moreover TPP also enhances catalitic activities of some enzymes by concentrating or dewatering proteins. This may be the result of increased flexibility of the enzyme molecule at TPP conditions. A noteworthy increase in the catalytic activity and yield of some enzymes obtained from TPP was reported (Akardere et al., 2010; Chaiwut et al., 2010; Duman and Kaya, 2013; Dogan and Tari, 2008;

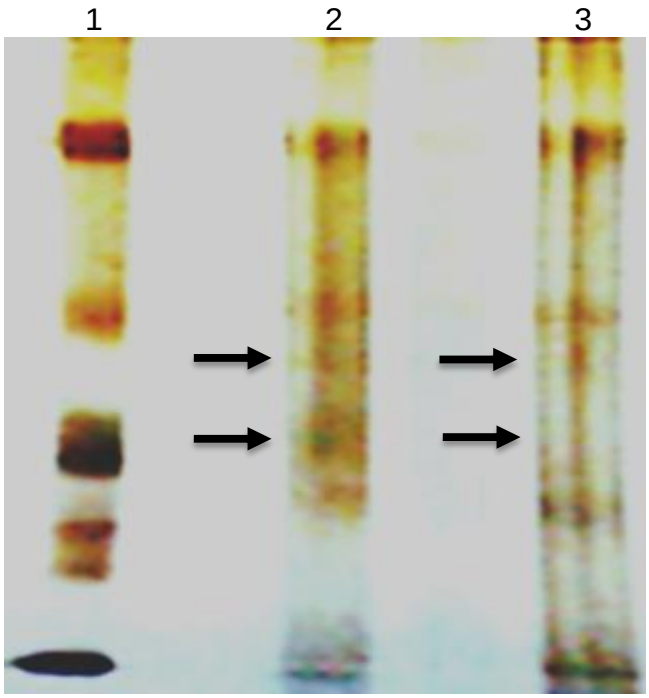

Figure 4. SDS-PAGE analysis of invertase from *M. charantia* [Lane 1; molecular weight markers (14-66 kDa), lane 2; crude extract, and lane 3; after TPP (medium phase)].

Sharma and Gupta, 2001a; Gagaoua et al., 2014, 2016).

In some studies two-step TPP was applied because of the low activity recoveries of one-step. Dhananjay and Mulimani have used the TPP as a two-step process to purify invertase from A. oryzae. In the first step, 61-fold purification with 1.4% recovery was achieved. The second TPP was resulted from 12-fold purification and 54% activity recovery (Dhananjay and Mulimani, 2008). Purification of laccase from Submerged cultures of *Ganoderma sp.* WR-1 (Rajeeva and Lele, 2011), purification of phospholipase D from *Dacus carota* (Sharma and Gupta, 2001b), and purification of protease from *Calotropis procera latex* (Rawdkuen et al., 2010) were also achieved by two-step TPP. In this study we planned to reveal the results of only one-step TPP for purification of invertase from bitter melon. The results show that while the purification fold (10.48) is better than some previous studies (Akardere et al., 2010; Chaiwut et al., 2010; Dhananjay and Mulimani, 2008; Duman and Kaya, 2013; Hussain et al., 2009; Liu et al., 2006), activity recovery (20.28%) is inadequate when only one-step TPP is used, and it may be increased by applying TPP again or other steps.

Characterization of invertase

SDS-PAGE analysis

A reduction in the number of protein bands in SDS-PAGE is evidence that some proteins have been eliminated. As shown in Figure 4, density of protein bands of the sample obtained from TPP application (lane 3) decreased according to crude extract (lane 2). So, simplier image in lane 3 is noteworthy which showes that *M. charantia* invertase had been partially purified by TPP.

Optimum temperature, pH and buffer concentration of M.charantia invertase

The effect of temperature on enzymatic activity of invertase presented in Figure 5 shows that the temperature at which the invertase activity is maximum is 50°C. Generally, invertases exhibit high catalytic activity in the temperature range of 35-75°C depending on the enzyme sources (Akardere et al., 2010; Tang et al., 1996; Kern et al., 1992; Persike et al., 2002).

The highest activity of invertase was found to be pH 5. As presented in Figure 6. Thus, we can conclude that *M.charantia* invertase is an acidic enzyme. The optimum pH values of invertases purified from different sources varies between 3.5 to 8.0 in the literature (Akardere et al., 2010; Lincoln and More, 2018; Tang et al., 1996; Persike et al., 2002 and Belcarz et al., 2002).

As shown in Figure 7, significant differences were observed in invertase activities at different buffer concentrations. Maximum activity of the enzyme was observed for 0.3 M pH 5.0 sodium acetate buffer solution.

Conclusion

The present study focuses on the purification and characterization of invertase from *M.charantia* using single step TPP and also optimization of the purification metod. Under different ammonium sulfate and t-butanol amount, different enzyme recoveries were obtained. And also incubation time was important parameter at this optimization procedure. Interfacial phase was containing the target protein. This work shows that, under optimized conditions, TPP is a useful method for partial purifiction or concentration of the invertase from bitter melon. We purified the enzyme using this technique partially. This technique has been fast and it is a very important feature for maintaining the enzyme activity; maybe more pure invertase can be obtained by applying the second or third TPP step under optimised condition in further sudies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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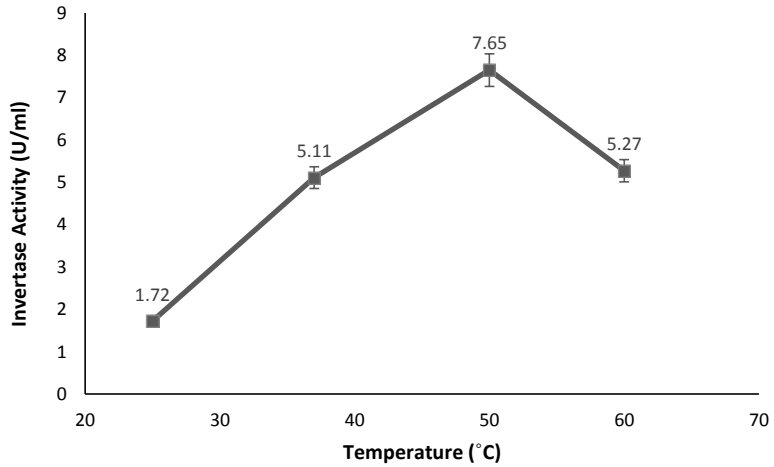

Figure 5. Determination of optimum temperature on invertase activity purified from *M. charantia*.

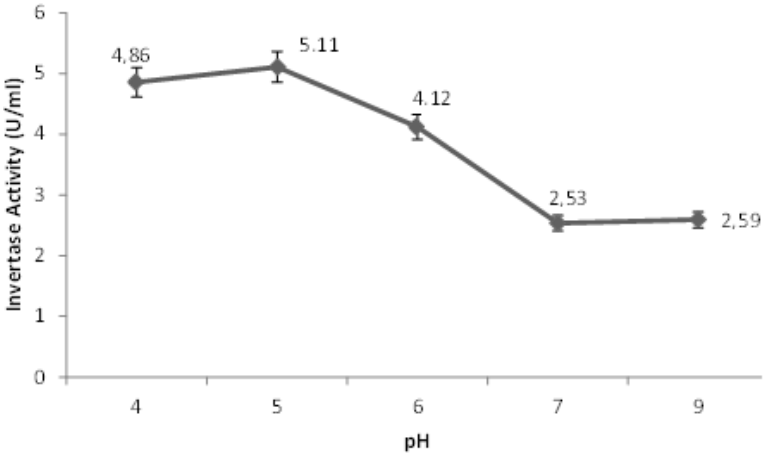

Figure 6. Determination of optimum pH on invertase activity purified from *Momordica charantia.*

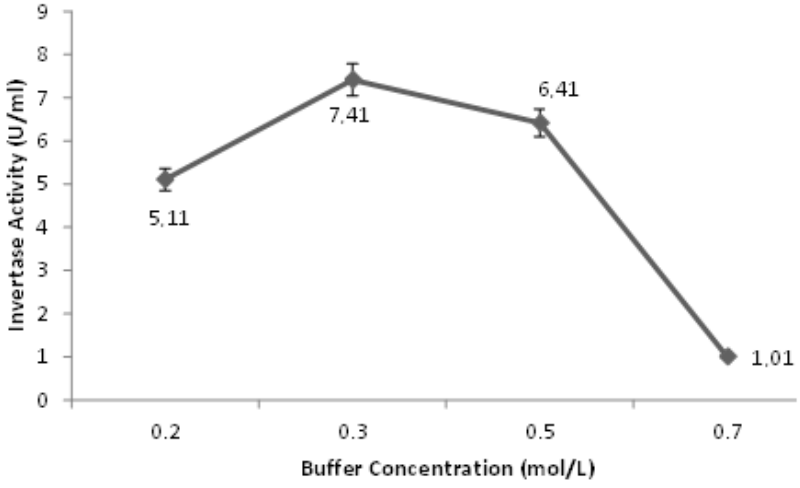

Figure 7. Determination of optimum buffer concentration on invertase activity purified from *M.charantia.*

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Cadmium-induced toxicity and antioxidant enzyme responses in tissues and organs of African catfish (*Clarias gariepinus*)

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Juveniles of *Clarias gariepinus* were exposed to different concentrations of cadmium chloride for 96 h under laboratory conditions using static bioassays with continuous aeration to determine its mean lethal concentration (LC_{50}), biochemical alterations, bioaccumulation and histological pattern in a sub-lethal toxicity test. The median lethal concentration (LC_{50}) at the end of the acute toxicity was 120.2 mgL^{-1}. Also the toxicant led to significant ($P<0.05$) changes in histotopathological parameters in the kidney, liver, gills and muscle as the toxicant concentration increased. The severity of these conditions was directly proportional to the toxicant concentration. Also, the biochemical studies showed that activities of superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST) ranged from (0.115 ± 0.15 to 1.634 ± 0.28) µmol/min/mg protein, (2.354 ± 0.45 to 7.734 ± 0.08) µmol/min/mg protein and (0.028 ± 0.05 to 0.21 ± 0.16) µmol/min/mg protein respectively. These values increased significantly ($p < 0.05$) with increase in concentration of cadmium chloride. The trend of bioaccumulation of cadmium in the tissues of the test organisms differs significantly ($p<0.05$) and it followed the order, kidney > liver > gill > muscle. The study concluded that cadmium is a potent pollutant that can cause severe damage in fish and hence man the final consumer.

Key words: *Clarias gariepinus*, biochemical alterations, bioaccumulation, static bioassays, histopathology.

INTRODUCTION

During last few decades, pollution of aquatic environment with heavy metal has been a worldwide problem which has necessitated considerable concern over its contamination and the potential health threat to public water sources. The non-degradable and persistent nature of the metal ions results in longer exposure and accumulation of these substances in the aquatic flora and fauna (Censi et al., 2006; Aladesanmi et al., 2014). When heavy metals enter water bodies, they alter water quality, bind to sediments and accumulate in aquatic biota causing anaemia, disturbance of physiological functions and mortalities of fish (Eichler et al., 2006; Aladesanmi et al., 2014). Heavy metal accumulation in the aquatic environment could result in toxicity to both aquatic life and humans. Edible fish present in aquatic bodies form an important group of organism as heavy metal once

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accumulated in fish tissues could act as a potential carrier of metal ion along the food chain. At the end, directly or indirectly the metal ion in the aquatic medium reaches to human. Gonzalez et al. (2007), defined metal bioaccumulation as the process whereby an organism concentrates metals in its body from the surrounding medium or food, either by absorption or ingestion. Heavy metals are of particular concern due to their toxic effect and ability to bio-accumulate in aquatic ecosystems body tissues and organs (Censi et al., 2006; Babalola et al., 2010). Several metals and their effects on the environment and human health have been studied. Cadmium, nickel and chromium among others have harmful effects on aquatic organisms as well as their final consumers. Cadmium is a non-essential element to all living organisms. Cadmium is a metal with an oxidation state of +2. It is chemically similar to zinc and occurs naturally with zinc and lead in sulphide ores (WHO, 2011). It is a soft white solid with a density of 8.64 g/cm^3. Rivers and lake shores are the areas primarily affected by diluted cadmium waste from industrial facilities in big cities (Randi et al., 1996). It is important to note that cadmium is a highly toxic element for all mammals and fish. Cadmium levels in fresh waters have constantly been on the increase in the last few decades and by contrast, the excretion of cadmium from living organisms is a slow process. In fish, cadmium can cause a number of structural and pathomorphological changes in various organs (Thophon et al., 2003). Cadmium is responsible for increased hypertension, emphysema, kidney tubule damage, impaired liver function, and cancer in mammals (Johri et al., 2010). The measure of a chemical's toxicity is its Median Lethal Dosage (LD50) value which is the concentration that can cause average kills of 50% of a test population of animals on trial. This is usually reported in milligrams of the chemical per kilogram of a test animal's life weight. Shuhaimi-Othman et al. (2010) reported the 96h-LD_{50} value of cadmium on *R. sumatrana* and *P. reticulata*, as 0.102 and 0.168 mg/L respectively. With *P. reticulata*, Park and Heo (2009) reported that 96h-LC50 for Cd as 30 mg/L. Gomes et al. (2009) also reported the 96 h-LC50 of cadmium on juvenile Brazilian indigenous fishes, curimata *Prochilodus vimboides* and piaucu 3.16 and 7.42 mg/L respectively.

Bio-monitoring of hazardous substances in tissues of aquatic organisms has been successfully applied during recent years for heavy metal pollution (Lamas et al., 2007). African sharp tooth catfish *Clarias gariepinus* has been reported to be a good bio-indicator. *Clarias gariepinus* is highly valued in Nigeria as it has the highest demand (1.5 million metric ton) and a per capital consumption of 7.5-8.5 kg annually (FDF, 2005) The African catfish is a vital part of inland water fisheries and a priceless source of protein for the native populations (Skelton and Teugel, 1992) Besides supplying protein for human consumption, *C. gariepinus* equally has medicinal usage in the treatment of eye problems and making concoctions for ante-natal purpose (Adesulu, 2007). Furthermore, *C. gariepinus* has been identified as a prime candidate for aquaculture as a food fish due to its hardiness, fast growth rate and large size attainable (Samuel and Ewa, 2000). It is also used in research and eco-toxicological studies to serve as biomarkers of environmental pollution and to evaluate the health of aquatic ecosystem (Kock et al., 1996; Nguyen and Janssen, 2002; Olaifa et al., 2003).

Accumulation of metals in different tissues viz., blood, gill, gut, liver, muscle, kidney, ovary and gonad etc., have been extensively investigated in various fishes. Most of these studies report metal accumulation indicating preference of the tissues for some metals over the others. The characterization of the accumulation of metals into different organs has proven to be a representative measure of the heavy metal exposure. Exposure to pollutants has caused major structural damages in the target fish organs, histological structure may change and physiological stress may occur. This stress also causes some changes in the metabolical functions (Van Dyk et al., 2008). It is generally believed that fresh water fish mainly accumulate cadmium in gill, liver and kidney. The gills are considered to be the most important uptake site for waterborne cadmium, whereas liver and kidney are the main storage and detoxification organs in fish (Reynders et al, 2006; Aladesanmi et al., 2014).

Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to toxins (Thophon et al., 2003). Toxicological evaluation in aquatics can also be carried out by using biomarkers. Biochemical markers like glucose, protein, and enzymes are frequently used as an indicator of the general state of health and early warning of stress in fish under unfavorable conditions (Barnhoorn and Van Vuren, 2004; Hamed et al., 2005; Osman et al., 2009). Therefore, the physiological changes observed serve as biomarkers of environmental pollution (Köck et al., 1996). These investigations have helped to understand the diversity in mechanism of heavy metal homeostasis in fishes although no universal mechanism could be established. The present study was however designed to determine the effect of sublethal concentration of cadmium on the tissues of *C. gariepinus*.

MATERIALS AND METHODS

Experimental design

One hundred and twenty juveniles *C. gariepinus* with mean weight of 25.45 ± 1.21 and mean length of 16.2 ± 0.24 cm were sourced from the fish hatchery at the Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife for the toxicity assay. The fishes were stocked in twelve 30 L plastic tanks each containing 10 L of dechlorinated tap water with each tank containing five fishes. They were acclimatized to laboratory conditions for one week in the stock tanks. The fishes were fed twice daily (12 h) with commercial

feed pellets 5% of their body weight. A static renewal bioassay procedure was adapted in which the test media was regularly renewed every 48 h at the set concentrations (Ayoola, 2008).

After acclimatization, a preliminary investigation (range finding test) was carried out prior to the commencement of the definitive test in duplicate (0, 60, 120, 180, 240 and 300 mg L^{-1}). Mortality was monitored daily and dead fish were removed immediately from the culture tank to avoid microbial contamination. The concentration of cadmium that caused 50% mortality in the fish population after 96 h was taken as the LC50 value using probit regression analysis (Niyogi et al., 2004). The fishes were not fed 24 h to the introduction of the toxicant as faecal matter and uneaten food may decrease the dissolved oxygen concentration and otherwise affect the biological activity of the toxicant.

For the sub-lethal exposure, 10% of 96-h LC_{50} was used to culture sixty fishes for 21 days. The sub lethal exposure consisted of two control tanks and five different concentrations (0, 15, 30, 45, 60 and 75 mg L^{-1}). Each with a replicate and containing five fishes per tank. A static renewal bioassay procedure was adopted in which the test media was regularly renewed every 48 h at the set concentrations to keep metal concentrations at minimal and to remove waste. The physical and chemical parameters (pH, dissolved oxygen (DO), conductivity and temperature of the experimental water were taken before and during the sub lethal exposure. The fishes were closely monitored observing behavioural changes twelve hourly. After 21 days, all the fishes were anesthetized by placing them in a refrigerator for six hours, sacrificed and the tissues were removed and prepared for probable histopathological examination, biochemical examinations and heavy metal analysis using standard protocols.

Stock solution

Anhydrous cadmium chloride ($Cdcl_2.21/2H_2O$) was used for the experiment because it is of low toxicity compared to the other forms of cadmium (Odiete, 1999). A stock solution of 1000 mg/L (1 g/L) of the cadmium was prepared by adding 1.0 g of cadmium to 1litre of distilled water. The amount of cadmium chloride which contained 1.0 g of cadmium was determined from the molecular and atomic weights as:

$$\frac{\text{Molecular weight of cadmium chloride}}{\text{Atomic weight of cadmium (Cd)}}$$

The different concentrations required were calculated as follows:

$$\frac{\text{Wt of cadmium required} \times \text{molecular wt of cadmium}}{\text{Atomic weight of cadmium}}$$

GST enzyme assay

The GST levels in response to Cd treatments were analyzed in the tissues using the method of Habig et al. (1974). Enzymatic assay was performed on the *C. gariepinus*, kidney, gill, liver and muscle. The tissues (50 mg) were homogenized in 50 mM Tris–HCl buffer, pH 7.4, and containing 0.2 M sucrose and centrifuged at 16,000 g for 45 min at 4°C. The pellet was discarded and the supernatant was used as the enzyme source. The reaction mixture in a volume of 3 ml contained 2.4 ml of 0.3 M potassium phosphate buffer (pH 6.9), 0.1 ml of 30 mM CDNB and 0.1 ml of 30 mM GSH, as enzyme source. The reaction was initiated by glutathione. The absorbances were read at 340 nm against the reagent blank. The results were expressed as µM/min/mg protein. The GST levels were measured using spectrophotometrically.

Catalase enzyme assay

Catalase levels in response to Cd treatments were evaluated by the method of Sinha et al. (1972). The tissues (50mg) were homogenized in 50 mM phosphate buffer, pH 7.0, and centrifuged at 16,000g for 45 min. The supernatant was used as the enzyme source. The reaction mixture contained 2 ml of phosphate buffer (pH 7.0) 0.45 ml H_2O_2, and 0.025 ml of enzyme source. The absorbance was read at 570 nm and the enzyme activity was expressed as micromoles of H_2O_2 consumed/min/ mg protein.

Superoxide dismutase enzyme assay

50 mg of the tissues was homogenized with 0.1 m phosphate buffer (pH 7.2), using a Teflon pestle over ice. The resulting homogenate was centrifuged at a speed of 5000 rpm for 10 min. To 20 µl of the homogenate, 250 µl of 75 mM of Tris–HCl buffer (pH 8.2), 30 mM EDTA and 30 µl of 2 mM of pyrogallol were added. An increase in absorbance was recorded at 420 nm for 3 min. One unit of enzyme activity brings about 50% inhibition of the rate of autooxidation of pyrogallol as determined by change in absorbance/min at 420 nm. The activity of SOD is expressed as units/mg protein (Weydert and Cullen, 2010; Mani et al., 2014).

Histopathological examinations

The Fish was decapitated, dissected and assessed individually by separating the experimental fish from the control fish. After proper dissection, the kidneys, the gills, liver and muscle were carefully removed and small pieces of the excised tissues were fixed in 10% formalin and embedded in paraffin wax at 56-58°C. The embedded tissues were then sectioned at 6 microns thickness, mounted and stained with haematoxylin and eosin for 2 to 5 mins. Each section was then used to make slides of tissue and then observed under the microscope for proper description of their histological structures, appearance, and cell arrangement. The respective photomicrographs of the slides were properly observed and interpreted (Kumar et al., 2005; Simonato et al., 2008).

Tissue metal analysis

At the end of the exposure period, kidney, gill, liver, and muscle, were dissected out. The tissues were oven dried at 60°C till constant weight. After determination of the dry weight the tissues were digested at 70°C in an aluminium block heater in screw capped polypropylene tubes with a mixture of 2 ml HNO_3 and distilled water (1:1 V/V). The heavy metal (Cd) contents of the tissue digests were determined using an Atomic Absorption Spectrophotometer (GBC- 902). Atomic absorption standards procured from Sigma were used in the analysis. The amount of metal accumulation was expressed as µg metal ion/g dry weight of the tissue. Based on the metal accumulation value, the term 'maximum accumulation factor' was derived. This represents ratio of highest metal accumulation in tissues of the effluent exposed fish and accumulation in the same tissues of the control fish.

Statistical analysis of the data was carried out using SPSS 21 version. Probit regression analysis was used to analyse the toxicological dose-response data involving mortality after 96 h. Data obtained were also subjected to correlation analysis to determine the relationship between the variables. One-way analysis of variance (ANOVA) was used to compare the means of results obtained from biochemical analysis and where a significant difference ($p < 0.05$) was obtained from the ANOVA, Duncan multiple range test (DMRT) was used to detect the source of the difference.

Table 1. Mean weekly physical and chemical parameters of experimental water during sub lethal exposure.

Concentration (mg/L)	pH	Temperature (°C)	Conductivity (µS/m)	DO (mg/L)
0	7.1±0.12	22.1±0.06	6.32±0.1	6.91±0.03
15	7.1±0.06	22.1±0.04	8.58±0.29	5.41±0.06
30	6.98±0.05	22.2±0.06	10.53±0.21	5.29±0.07
45	6.93±0.05	22.56±0.05	13.08±0.79	4.62±0.18
60	6.88±0.1	23.05±0.06	17.05±0.21	4.38±0.17
75	6.8±0.01	23.9±0.1	21.81±0.72	3.9±0.13

Table 2. Acute toxicity median lethal concentration test results.

Probit regression equation					Chi-square values		
H	LC50	Lower	Upper	Y= a-bx	Observed	Table	Sig (0.05)
24	204.41	2.21	17.09	-22.29-bx	5	1.19	0.754[a]
48	151.18	1.81	11.28	-14.26-bx	5	3.19	0.363[a]
72	128.73	1.79	10.23	-12.67-bx	5	2.487	0.478[a]
96	120.2	1.79	12.17	-14.52-bx	5	0.812	0.847[a]

RESULTS

Physical and chemical parameters

The pH, DO, temperature, conductivity values of the experimental water recorded before start of the experiment include 7.0, 22.4, 6.2, 6.75 and 6.0 respectively. The weekly values of physical and chemical parameters of the experimental water monitored for different cadmium concentrations and the control are shown in Table 1. The result therefore revealed there was no significant ($p<0.05$) variation between the control group and the groups treated with cadmium. The mean weekly pH value of the ranged from 6.8 ± 0.01 - 7.1 ± 0.12. The highest mean weekly temperature recorded ranged from 23.9 ± 0.1 - 22.1 ± 0.06°C. The result obtained for the mean water temperature showed that there was no significant ($p<0.05$) variation in the temperature between the control group and other groups with varying concentrations of cadmium throughout the experimental period. The mean weekly value of conductivity ranged from 6.32 ± 0.1 - 21.8 ± 0.72 µS/m. A significant increase was observed in mean conductivity as cadmium concentration increased. The mean weekly value of dissolved oxygen (DO) ranged from 6.91 ± 0.03 - 4.38 ± 0.17 mg/L. The results obtained further showed a significant ($p< 0.05$) variation between the control group and the group with the highest concentration of Cadmium (Table 2).

Behavioural response

During the 96 h acute toxicity test, mortality was recorded in all of the treatment groups except the control and the group treated with the least concentration (60 mg/L). All the fishes in the highest concentration (300 mg/L) as well as two of the fishes in the 180 mg/L and four of the fishes in the 240 mg/L died within 24 h of exposure. The remaining fishes in the 240 mg/ L treatment group died within 48 h of exposure, with 50 and 67% death recorded in treatment groups with 120 and 180 mg/L concentrations respectively after 72 h exposure. However, mortality levels rose from 0 to 100% across the groups as shown in Figure 1. The median lethal concentration obtained after 96 h was 120.2 mg/L as shown in Table 3.

The activity of GST in all tissues ranged from 0.028 to 0.21 µmol/min/mg protein. GST activity was highest in the liver across all treatment groups except in the 60 and 15 mg/L treatment group as seen in Figure 1. The liver of the 75 mg/L treatment had the highest activity. GST activity was also high in the kidney across the concentration when compared to the kidney in the control group with the exception of the 30 mg/L which had the highest activity. There was also increase in GST activities in the gills and muscles as the concentration increased as shown in Figure 2. The activity of SOD ranged from 0.115 to 1.634 µmol/min/mg protein. SOD activity increased in the tissues across the treatment groups when compared to the control treatment though the activity was highest in the liver across treatment groups with the exception of the 30 mg/L treatment group as seen in Figure 3. It is also well expressed in the muscle across the treatment groups as shown in Figure 4. The activity of CAT in all tissues ranged from 2.354 to 7.734 µmol/min/mg protein. CAT activity was highest in the liver across all treatment groups (Figure 4). The histopathology

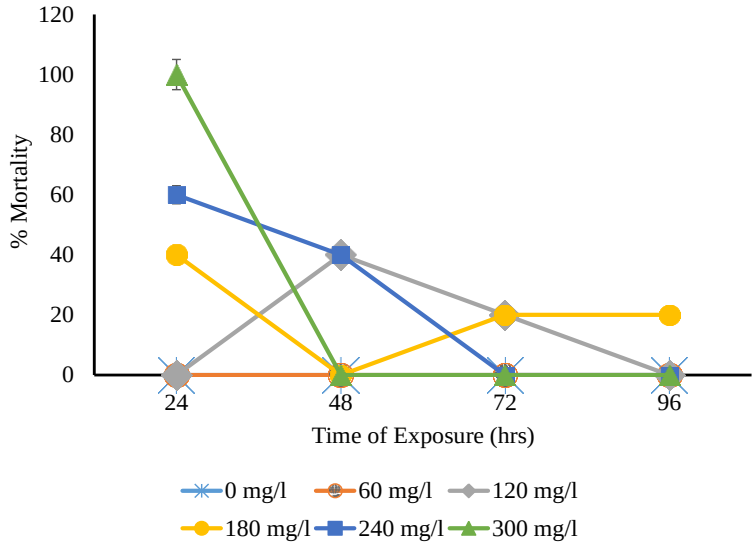

Figure 1. mortality of fish with length of exposure to cadmium during acute test.

Table 3. Behavioural changes observed in fish acute toxicity study.

Exposure time / Concentration (mg/L)	24 h						48 h						72 h						96 h					
	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
Loss of reflex	-	-	-	-	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+
Moulting	-	-	-	-	+	+	-	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Discolouration	-	-	-	-	-	+	-	-	-	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+
Air gulping	-	-	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+
Erratic swimming	-	-	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Barbel deformation	-	-	-	-	-	+	-	-	-	-	+	+	-	-	+	+	+	+	-	-	+	+	+	+
Mucus secretion	-	-	-	-	+	+	-	-	-	-	+	+	-	-	+	+	+	+	-	-	+	+	+	+

Keys: += Present, - = Absent, A (0 mg/L), B (60 mg/L), C (120 mg/L), D (180 mg/L), E (240 mg/L), F (300 mg/L).

of different *C. gariepinus* tissues revealed that there are several changes in the different tissues (muscle, liver, gills, and Kidney) of the fish subjected to sublethal concentrations of cadmium as shown in Plates 1, 2, 3 and 4.

In the present study, cellular necrosis and blood congestion were common to all the tissues and organs. The photomicrograph of the liver of the control fish showed normal architecture with hepatocytes presenting a homogeneous cytoplasm as could be seen in Plate 1a. However, extensive necrosis and destruction of liver hepatocytes were observed in the 60 and 75 mg/L treatment group. Other liver anomalities such as cytoplasmic vacuoation blood congestion, hypertrophy; edematous fluid, and cellular necrosis were also observed across the treatment groups as seen in Plates 1.

The photomicrograph of the kidney of the control fish showed a typical structural organization of the kidneys with hepatocytes presenting a homogeneous cytoplasm as could be seen in Plate 2a. However, extensive necrosis and destruction of liver hepatocytes were observed in the 60 and 75 mg/L treatment group. Other kidney anomalies such as dilation of Bowman's Space, cellular degeneration, infiltration of edematous fluid, glomerular expansion, and increase in the diameter of renal tubules were observed across the treatment groups as seen in Plate 2. The white and red muscles were found to be intact in histological architecture of the control group showing the presence of normal myotomes with equally stained muscle bundles. The fishes exposed to all the effluent treatment showed necrosis of the muscle bundle with it being more severe in the 60 and 75 mg/L treatment. It further showed, disorganization of the muscle bundle, inflammation, hypertrophy, edema and necrosis (Plate 3). The control gills appeared normal in form and histological architecture and revealed the appearance of the gill arch, lamellae with blood spaces. The primary lamella base had an even sparsely distributed network of similar sized cells. Blood

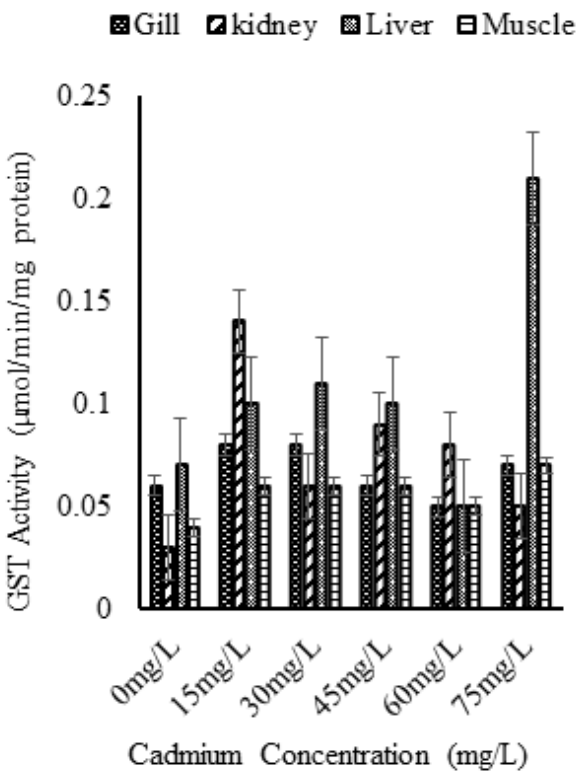

Figure 2. Glutathione S- transferace activity in tissues and organ of *C. gariepinus*

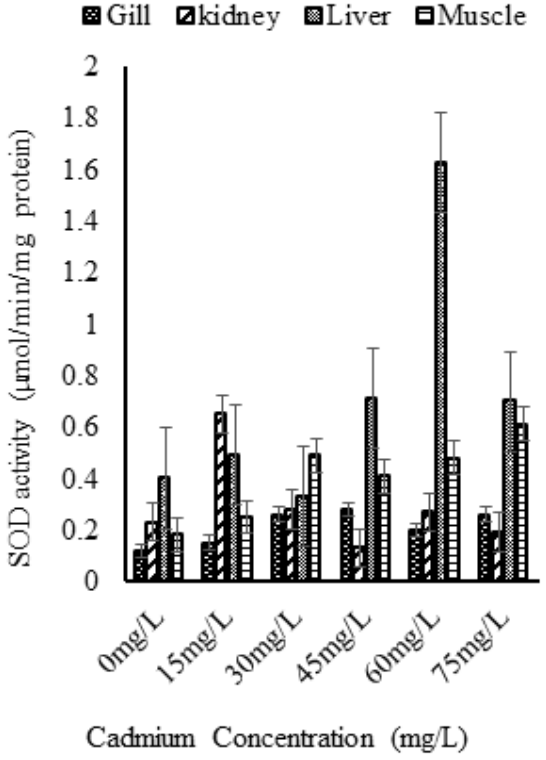

Figure 3. Superoxide dismutase activity in tissues and organ of *C. gariepinus*.

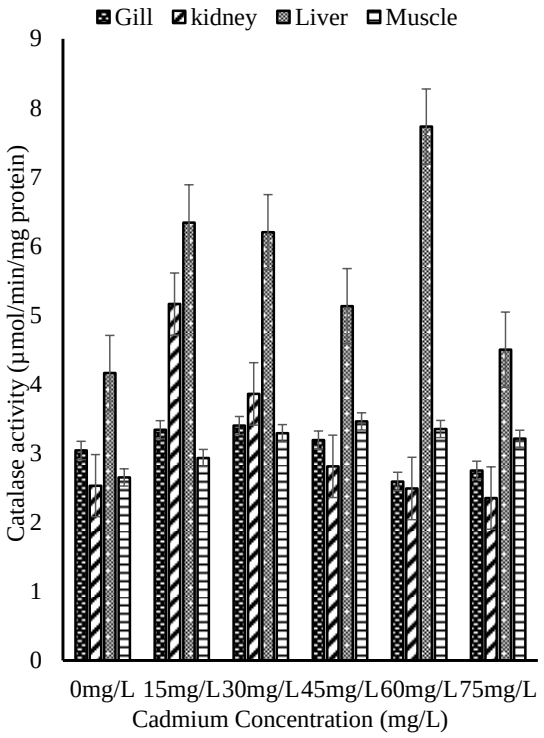

Figure 4. Catalace activity in tissues and organ of *C. gariepinus.*

congestion; telangiectasis; severe hypertrophy, fused lamellae, vacuolation, epithelium were histological alterations observed across the treatment groups (Plate 4).

Accumulation of cadmium in the tissues/organs of *C. gariepinus*

Figure 5 shows the accumulation of cadmium in the gill, liver, kidney and muscle of *C. gariepinus* exposed to different concentrations of cadmium. The result revealed that there was a progressive increase in the concentration of cadmium in the test fish. Kidney was found to have highest accumulation of cadmium in all the experimental tanks (Figure 5). Comparative analysis showed that tissues of fish in 60 mg/L (E) was statistically higher than other levels of concentrations. The result also showed that there were consistent increase in the order of accumulation of cadmium in the kidney, liver, gill and muscle of juvenile *C. gariepinus*.

DISCUSSION

The results of the physical and chemical analysis of the water of this study showed that there was slight dose-dependent decrease in pH and concentration of dissolved oxygen, and a slight dose-dependent increase in

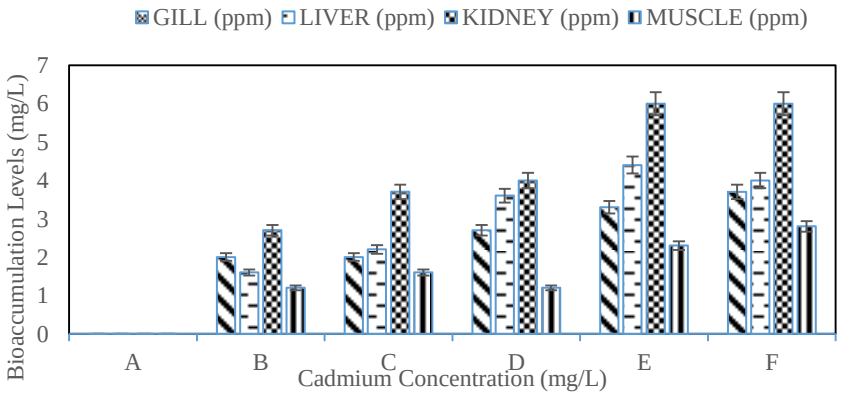

Figure 5. Bioaccumulation in *C. gariepinus* exposed to varying concentration of cadmium.

temperature and conductivity across the different concentrations. Temperature has a profound effect on biological processes (Cossins, 2012); the metabolic activities of aquatic organisms increase with temperature (Adamu and Solomon, 2015). Research has shown that the normal range of temperature in the tropics to which fish are adapted is 22-35°C (Adeyemo et al., 2003). The mean temperature recorded during this study was within WHO limit for fresh water fishes. In addition, there was slight increase in temperature as the concentration of cadmium increased. Similarly, the conductivity of the culture water was observed to increase as cadmium concentration increased. This could be attributed to the release of waste from fish and the nutrients composition of the feed (Lawson, 2011).

Research has shown that suitable water quality for any fish culture in the tropical region must have dissolved oxygen of at least 3 mg/L (Colt, 2006). The mean dissolved oxygen contents recorded in this study was greater than 3 mg/L in all the treatment group. In this study, dissolved oxygen of the culture media decreased with increase in concentration of cadmium and this corresponded with the result of Khalid who observed a decrease in dissolved oxygen concentration with increase in the level of nickel in the culture water (Al-Ghanim, 2011).

Alteration in behaviour is considered as a sensitive biomarker to evaluate the toxicants exposure/ effect (Gerhardt, 2007). The studies on fish behaviours provide lots of knowledge and information because behavioural alteration can be related to physiological biomarker in aquatic species (Sabullah et al., 2015). For example, the monitoring of behavioural response becomes an impending option to environmental change, disease, stress and the presence of toxic compounds in water, which in most condition initiates the variation of fish behaviour (Gerhardt, 2007). The behavioural changes recorded during the acute exposure of juvenile *C. gariepinus* to varying concentration of cadmium include; loss of reflex, moulting, discolouration, air gulping, erratic swimming, barbell deformation and excessive mucus secretion. The control fish was used as standard against the experimental fish to monitor behavioural changes. The gradual changes observed at lower concentration of cadmium in the fish behaviour reflected a transient stress induced osmotic imbalance. However, deep behavioural changes observed in fish exposed to higher concentrations of cadmium showed that cadmium could induce stress. Studies have shown correlations between behavioural and physiological indicators of toxicity and have therefore succeeded in eliminating the complicating effects faced when comparing different behavioural and physiological studies. Studies are beginning to correlate physiological changes induced by toxicant exposure with behavioural disruption, thus providing ecological relevance to physiological measures of toxicity (Scott and Sloman, 2004).

The 96 h LC_{50} value obtained for juvenile *C. gariepinus* exposed to varying concentrations of cadmium in this study was 120.2 mg/L. This value falls within range of cadmium concentrations that have been reported in a number of fish species as: 173.78 mg/L in *Rita rita* (Ghosh and Mukhopadhyay, 2000), 121.8 mg/L in *Cyprinius carpio* (Muley et al., 2000) and 17.9 mg/L in *Cherax tenuimanus* (Chambers, 1995).

Antioxidant enzymes are capable of stabilizing, or deactivating free radicals before they attack cellular components. Antioxidants enzymes are involved in the detoxification of both xenobiotics as well as endogeneous reactive compounds of cellular metabolism. They act by reducing the energy of the free radicals or by giving up some of their electrons for its use; thereby causing it to be stable. They may also interrupt with the oxidizing chain reaction to minimize the damage caused by free radicals (Magder, 2006). A number of authors have shown that several biomarkers of oxidative stress can provide satisfactory information on the response of fish to environmental stressors (Achuba and Osakwe, 2003;

Farombi et al., 2007). GST activities in this study revealed that the tissues exposed to higher concentrations of cadmium had higher activities when compared to the activities of the control fish (Guthenberg and Mannervik, 1981). Elevated levels of antioxidant defence enzyme systems superoxide dismutase, catalase and glutathione s transferace may be due to the fact that under oxidative stress, the toxic effects of pollutants may trigger the production of antioxidants defences to overcome stressful conditions generated by such pollutants (Bebianno et al., 2004). SOD and CAT, provide an important means of cellular defences against free radical damage, therefore, this could be responsible for higher activities as observed in this study.

Cadmium has been reported to possess nephrotoxic action in man and various animals. In fact, kidney is the principle target organ of cadmium toxicity and chronic cadmium exposure in almost all animal species is characterized by varying degree of renal damage (El-Sokkary et al., 2009; Kumar and Singh, 2010). Necrosis of epithelial cells of renal tubules, glomerular contraction and reduction of Bowman's space were observed in the exposed fish.

The liver of fish is very sensitive to environmental contaminants and because many contaminants tend to accumulate in the liver, it hereby exposes it to a higher risk than other organs in a polluted environment (Heath, 1995). These findings were apparent as the liver is considered the organ of detoxification and excretion. The results of this study showed liver degeneration of the hepatocytes, congestion of central vein, area of necrosis, cytoplasmic vacuolation, vascular dilation and dilation of sinusoids in the hepatic cells in exposed fish as compared to that of the control fish. These results were in accordance with those reported by (Mela et al., 2007; Van Dyk et al., 2007).

Gill is the first direct contact with water from the external environment and changes in the fish gill is the most usually distinguished reactions to environmental toxins (Van der Oost et al., 2003). Fish gill defence mechanism and it potential as biomarker has been well explained (Nascimento et al., 2012). It was also reported that necrosis and desquamation of gill epithelium as well as lamellar curling and aneurisms were the direct deleterious effects reported in chronic lead exposed to *C. gariepinus* (Olojo et al., 2005). This present study revealed structural deformation such as epithelial lifting at secondary lamella, hyperplasia of primary epithelium, fussion of secondary lamella, necrosis and blood congestion. Furthermore, it was observed that *C. gariepinus* showed apparent histological changes such as thickening, necrosis of the muscle bundle, intermuscular edema, necrosis of the muscle bundle, blood congestion; disorganization of the muscle bundle, inflammation, hypertrophy, and edema. Histopathological changes in the muscle was dependent on the concentration of the toxicant and was visible in this study as the concentration increased. The analysis carried out on the tissues/organs (liver, kidney, gill and muscle) using Atomic Absorption Spectrophotometer (AAS) showed a significant difference ($p<0.05$) in the cadmium concentrations across the tissues/organs of *C.gariepinus*. The studies revealed that there was a progressive increase in the concentration of cadmium in the tissues of *C. gariepinus*. Muscle was found to have the lowest accumulation of cadmium in all the experimental tanks similar to the result of (Zhang et al., 2007) in the study of enhanced bioaccumulation of cadmium in Carp. Comparative analyses showed that tissues of fish in 75 mg/L was statistically higher than other level of concentrations. The kidney had the highest bioaccumulation followed by the liver, gill and muscle. This result corresponded with the results of other authors who reported that cadmium accumulates in tissues of carp *Cyprinus carpio* in following order: kidney> liver> gills (De Smet et al., 2001). (Kumar et al., 2007) have also reported similar accumulation pattern in *Clarias batrachus* in an experimental study. Kidney is the prime target organ for cadmium. The liver also stores a considerable part of the accumulated cadmium. (Aladesanmi et al., 2014) reported the distribution pattern of some heavy metals in *C. gariepinus* in Ilesha and Oshogbo in the order of liver > gills > muscle > fin. This study also revealed that with 21 days of exposure to cadmium, the fish most especially those exposed to the highest concentration bioaccumulated the metal beyond the threshold recommendation level of 0.5 mg/L (FAO/WHO, 1984; FAO/WHO, 2011).

CONCLUSION AND RECOMMENDATION

The results obtained from acute toxicity studies shows that cadmium is toxic to *C. gariepinus* at 120.2 mg/L, and that the toxic response in the fish was dose and duration dependent. However, the combination of this method improves the understanding of the biological risk on aquatic life arising from heavy metal contamination.

The response of the antioxidant enzymes (GST, SOD and CAT) were found to be dependent on concentration and duration of exposure and hence, could be considered as sensitive biomarkers for biomonitoring the aquatic environment contaminated with cadmium. Alterations in the histology of *C. gariepinus* tissues obtained from this results provide evidence to support the use of pathological changes in fish as an indicator for monitoring the effect of exposure to toxicants which are capable of altering the biochemical profile of an organism. Therefore, the consumption of cadmium contaminated fish may pose serious health risks to fish consumers. It can be conclusively deduced from this study that fish has the tendency to bioaccumulate heavy metals in a polluted environment. Hence, the indiscriminate consumption of fish from a polluted water body should be discouraged.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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