Niger. J. Physiol. Sci. 34 (December 2019) 215-221
www.njps.com.ng

Niger. J. Physiol. Sci.

Cardiorenal Effects of Pharmaceutical Plant Effluent in Mice (*Mus musculus*)

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Summary: Many pharmaceutical industries carelessly handle their effluents and indiscriminately release same to aquatic environment. These effluents often find their ways into surface and ground waters, contaminating public water and thus, serving as a potential threat to animals and human health. In this study, we investigated the cardiorenal effects of chronic oral exposure to pharmaceutical effluent in mice. Thirty male mice (*Mus musculus*) were randomly divided into groups A-F and treated with 0.2 mLs 0.0 %, 2.5 %, 5.0%, 10.0%, 20.0% and 40% concentration (v/v, effluent/distilled water) of the effluent for 28 days, respectively. At the end of the experiment, the animals were sacrificed by cervical dislocation. Activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were determined in serum and heart homogenate, while uric acid, creatinine and electrolytes (sodium, potassium, bicarbonate and chloride ions) were determined in serum only. Data were expressed as Means ± standard error of mean and values were considered significant at $p < 0.05$. Results showed that, oral exposure to pharmaceutical effluent reduced ($p < 0.05$) cardiac ALP, AST and ALT activities as well as serum ALT activity. However, serum activities of ALP, creatinine and uric acid were elevated ($p < 0.05$). Similarly, there was derangement of electrolytes (potassium, chloride, bicarbonate and sodium ions) in the exposed mice, compared with control. This study has demonstrated that poorly treated pharmaceutical effluent disrupted cardiac and serum enzyme activities, caused electrolytes imbalance and elevated serum uric acid level, suggesting that, drinking water contaminated with pharmaceutical effluent may impair kidney and cardiac functions. Further study, investigating the histology of the kidney and heart of the pharmaceutical effluent-exposed animals as well as mechanism(s) of cardiorenal toxicity of the effluent, should be carried out to exploit its roles in pathogenesis of cardiorenal diseases.

Keywords*:* cardiorenal, pharmaceutical effluent; electrolytes; enzymes; *Mus musculus*

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Manuscript Accepted: November, 2019

INTRODUCTION

Extensive industrialization, due to increasing population density and high urbanized societies, has resulted to global waste water management problem (Akpor et al., 2014). Many industries indiscriminately discharge contaminated and untreated waste products in to aquatic environment, causing pollution (Abu, 2012; Oyeniyi and Latunji, 2012). Inlands and estuary waters are both vulnerable to pollutants from industrial effluent discharge, affecting quality of water supply for domestic use (Osibanjo et al., 2011). This, therefore, causes global decrease in quality of drinking water below WHO standard and accounts for 3.1% death increase, globally (Khan et al., 2013; Pawari et al., 2015). It has also been reported that, heavy metals in water pollutants induce formation of reactive oxygen species (ROS), leading to organ damage in animals (Bando et al., 2005; Adegbesan and Adenuga, 2007).

Pharmaceuticals are among the important pollutants commonly released into environment (Stackelberg et al., 2004). Pharmaceutical industries generally produce large quantity of wastes during manufacturing and maintenance operations, which are subsequently released into water bodies (Chander et al., 2014). These wastes often consist of toxic biodegradable and non-biodegradable substances, and therefore remain public health concern. Although, concentration of pharmaceuticals discharged into environment is drastically reduced by various physical and biological processes occurring in aquatic ecosystem, trace concentrations of human and veterinary pharmaceutical compounds as well as their metabolites have been detected in water bodies like surface, ground and drinking waters (Kolpin et al., 2002; Benotti and Brownawell, 2009; Bruce et al., 2010). Exposure to trace amount of pharmaceuticals for a long time can result in considerable adverse effects, such as tissue damages and inhibition of cell proliferation, in humans and aquatic life (Brooks et al., 2003; Pomati et al., 2006).

In Nigeria, there is continuous increase in pharmaceutical industries, mostly located in riverside

areas. The discharge practices of these industries are too crude, putting society in danger, especially in the industrialized parts of the country (Ijeoma and Achi, 2011). The industries carelessly handle their wastes and indiscriminately discharge their effluents into water bodies, promoting aquatic pollution and affecting human and environmental health (Osaigbovo and Orhue, 2006; Idris et al., 2013). This is largely due to their ignorance of harmful effects of xenobiotics and microbes present in the effluent on both aquatic and terrestrial life as well as poor enforcement of stringent regulations prohibiting illegal discharge of effluents (Lateef et al., 2007; Adeoye et al., 2015).

Previous studies have revealed the mutagenicity, genotoxicity and hepatotoxicity of pharmaceutical effluent (Zhao *et al.*, 2007; Akintonwa *et al.*, 2009; Bakare *et al.*, 2009; Adeoye *et al.*, 2015) but there is paucity of information on its cardio-renal toxicity. Recently, we showed that oral exposure to pharmaceutical effluent impairs cardiac Na^+/K^+-ATPase activity and reduced cardiac weight index in mice (Abdulkareem et al., 2019). In this present study, we evaluated the effects of chronic oral exposure to pharmaceutical effluent on both renal and cardiac functions in mice.

MATERIALS AND METHODS

Effluent collection: The raw effluent from a pharmaceutical plant (which produces analgesics, anti-malarias, anesthetics, multivitamins and antibiotics) in Ilorin, Kwara State, Nigeria was collected in a 5L transparent plastic container (from the point of discharge into the environment). The collected effluent was filtered; the pH was taken and it was stored at 4°C until use.

Physico-chemical properties and heavy metal analysis: The effluent was analyzed for a number of standard physico-chemical properties, including: chemical oxygen demand (COD), total dissolved solids (TDS), alkalinity, biochemical oxygen demand (BOD), chlorides, nitrates, ammonia, and phosphates, according to methods described by APHA (1998). Eight metals which include: cadmium (Cd), chromium (Cr), iron (Fe), zinc (Zn), nickel (Ni), manganese (Mn), copper (Cu), and lead (Pb) were analyzed in the effluent sample according to standard analytical methods as previously reported (Bakare et al., 2009). Concentration of the metals were estimated by using an Atomic Absorption Spectrophotometer (Perkin Eelmer E., Analyst, 2000, USA).

Animals and experimental design: Thirty male mice (*Mus musculus)* of 8-10 weeks old were obtained from Central Research Laboratory, University of Ilorin, Ilorin, Nigeria. They were kept in a clean, frequently disinfected and well-ventilated animal house of the Department of Zoology, University of Ilorin, for 2 weeks in order to acclimatize. All mice were maintained under standard condition (12h light: 12h dark) and were exposed to standard feed and drinking water *ad libitum.* Handling of animals was kept in accordance with the regulation of University of Ilorin Ethical committee and in conformity to the NIH Guidelines on the care and use of laboratory animals. The mice were divided into 6 groups. Group A (control) mice received 0.2ml distilled water, while groups B-F were treated with 0.2 mLs 2.5%, 5.0%, 10.0%, 20.0% and 40% concentration (v/v, effluent/distilled water) of the effluent, respectively. All the treatments were administered orally and lasted for 28 days.

Tissue preparation: On 29_{th} post-treatment day, the animals were sacrificed by cervical dislocation. The heart was quickly excised, cleared of connective tissues, and transferred into the ice-cold 0.25M sucrose solution. The heart was homogenized in ice-cold 0.25M sucrose solution (1:5, w=v) as previously reported (Olatunji et al., 2006). The resulting homogenates were kept frozen overnight and centrifuged before use.

Enzymes assays: Method of Reitman and Frankel's (1957) was employed in estimating alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities. Activity of alkaline phosphatase (ALP) was determined, using Babson et al. (1966) method.

Determination of serum uric acid and creatinine concentrations: Concentrations of serum uric acid and creatinine were determined, using the method of Tietz (1994) as outlined in Randox kits, UK.

Determination of serum electrolytes: Potassium, sodium and chloride were determined by the method of Tietz *et al.* (1996), while serum bicarbonate level was assessed by the method of Roth and Chan (2001).

Statistical analysis

All data were expressed as means ± standard error of mean (SEM). Statistical analysis was performed with Graphpad Prism 5 (GraphPad Software, USA). Mean values of variables among the groups was compared by One-way analysis of variance (ANOVA), following Bonferroni Post-*hoc* test. Values were considered significant at $p < 0.05$.

RESULTS

Physicochemical properties of the raw pharmaceutical effluent: Details of the physicochemical properties of the effluent have been provided in our previous study (Abdulkareem et al., 2019). The pH value (6.40) of the effluent falls within national permissible limit (NESREA). Concentrations

of Cadmiun (Cd), Chromium (Cr), Zinc (Zn), and Copper (Cu), phosphate, alkalinity and TDS were lower than international (USEPA) and national (NESREA) recommended limits, while iron (Fe), manganese (Mn) and ammonia (NH_3) in the sample were higher than the limits. Lead (Pb) and nickel (Ni) were below detectable limits.

Cardiac and serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP): As shown in figures 1, 2 and 3), analyses of enzyme activities showed that, 28 day oral administration of pharmaceutical effluent significantly decreased ($p < 0.05$) cardiac activities of ALP, AST and ALT. Similarly, there was a significant decrease ($p < 0.05$) in serum activity of ALT in the exposed mice. In contrast, there was an increase ($p < 0.05$) in serum activity of ALP while serum activity of AST was not significantly changed ($p > 0.05$), when compared with control.

Serum creatinine and uric acid: Figure 4 shows that, chronic oral exposure to pharmaceutical effluent at 5 %, 10 %, 20 % and 40 % concentrations significantly ($p < 0.05$) elevated serum uric acid and creatinine levels, as compared with control.

Serum electrolytes: There was a significant ($p < 0.05$) concentration independent increase in serum potassium, chloride and bicarbonate ions values, meanwhile values of sodium ion decreased (concentration independent) significantly ($p < 0.05$) as compared with control (Table 1).

Fig. 1: Effect of pharmaceutical plant effluent on cardiac and serum alkaline phosphatase (ALP) activities in mice. Oral exposure to the effluent significantly decreased cardiac ALP activity, but increased the activity in the serum (*$p < 0.05$ vs control).

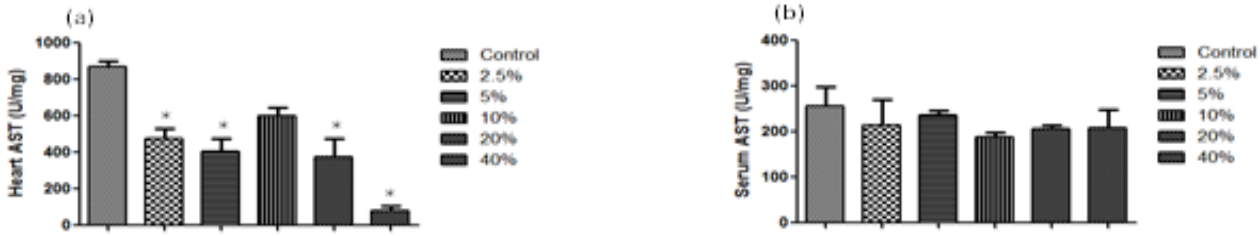

Fig. 2: Effect of pharmaceutical plant effluent on cardiac and serum aspartate aminotransferase (AST) activities in mice. Oral exposure to the effluent significantly decreased cardiac AST activity, whereas, there was no significant increase in serum activity of the enzyme (*$p < 0.05$ vs control).

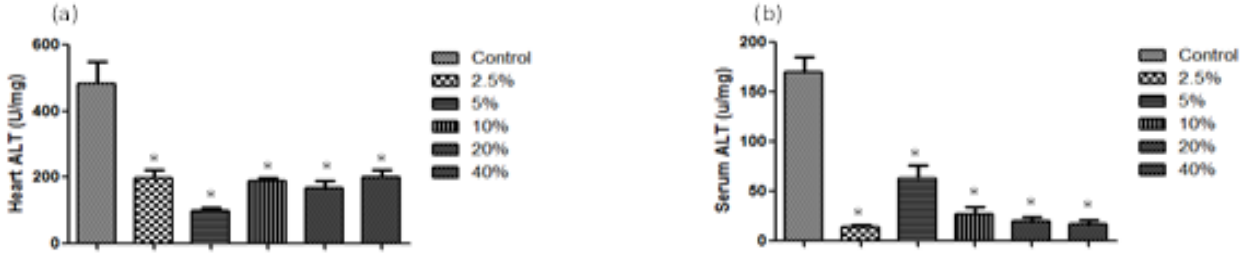

Fig. 3: Effect of pharmaceutical plant effluent on cardiac and serum alanine aminotransferase (ALT) activities in mice. Oral exposure to the effluent significantly decreased cardiac ALT activity, and elevated serum activity of the enzyme (*$p < 0.05$ vs control).

Fig. 4: Effect of pharmaceutical plant effluent on serum uric acid and creatinine in mice. There was an increase in serum uric acid and creatinine levels at concentrations 5 %, 10 %, 20 % and 40 % (*p < 0.05 vs control).

Table 1:
Effect of pharmaceutical effluent on electrolytes

Treatment Groups	POTASSIUM	SODIUM	CHLORIDE	BICARBONATE
Control	4.63±0.89$_a$	73.38±3.82$_a$	13.07±0.46$_a$	3.71±0.92$_a$
2.5 %	9.60±0.72$_b$	73.17±0.35$_a$	15.31±2.87$_{ab}$	17.94±9.32$_b$
5 %	6.12±1.18$_{ab}$	60.19±3.87$_b$	21.82±2.70$_{bc}$	17.66±3.50$_b$
10 %	6.45±1.50$_{ab}$	57.90±0.35$_b$	24.51±2.25$_c$	10.08±3.02$_{ab}$
20 %	6.86±1.05$_{ab}$	47.21±2.43$_c$	16.81±1.92$_{ab}$	11.72±7.24$_b$
40 %	4.30±0.81$_a$	58.35±2.01$_b$	15.44±1.67$_{ab}$	6.70±3.24$_a$

Values along the same column with different superscripts are significantly different (p < 0.05)

DISCUSSION

The results of this study show that, chronic oral exposure to pharmaceutical effluent can induce cardiac and renal dysfunctions by disrupting enzyme activities, raising serum uric acid and by causing electrolytes imbalance. Activities of ALP, AST and ALT in both tissues and serum are important biomarkers in determining organ integrity and function (Arise et al., 2012; Shahjahan et al., 2004). More so, correlation of ALP with C-reactive protein, inflammation, obesity, and atherosclerosis makes it a potential diagnostic marker or predictor of cardiovascular disease (Webber et al., 2010). During organ damage, these enzymes are leaked into the blood, causing increase in their serum levels, with subsequent decreased activities in the respective organs (Arise et al., 2012). Therefore, decrease in cardiac ALP, AST and ALT with concomitant elevated level of serum ALP indicates that, heart membrane was damaged, after chronic exposure to pharmaceutical effluent, and probably resulting in cardiac dysfunction. Decrease in cardiac AST and ALT activities was equally followed by a decrease in serum activity of the enzymes. This may mean that, the decrease in cardiac enzymes activities observed, was due to antagonistic effect of the effluent on AST and ALT production (Akanji et al., 1993). Previous studies have associated elevated serum ALP level with coronary artery disease (CAD), as it promotes vascular calcification through pyrophosphate pathway (Johnson et al., 2006; Schoppet and Shanahan, 2008). Furthermore, high level of serum ALP increases risk of mortality and unfavourable prognosis in coronary artery disease (Park et al., 2013; Wannamethee et al., 2013) as well as other cardiovascular diseases (CVDs) such as peripheral artery disease (Cheung et al., 2009), left ventricular hypertrophy (Nasri et al., 2004), secondary cardiac failure and diastolic dysfunction (Salgueira et al., 2005). Increase in serum ALP level observed in our study, therefore, suggests that, prolonged drinking of pharmaceutical effluent-contaminated water may promote the incidence of CVDs. Our finding is in consistence with our previous study (Abdulkareem et al., 2019) and the report of Karabulut et al. (2014).

Uric acid (UA) is a double bonded organic compound which, due to its antioxidant property, protects against oxidative stress (Sautin and Johnson, 2008). Elevated level of this compound however, may serve as an independent risk factor for coronary artery disease as it is frequently observed in patients with heart failure (Tian et al., 2012). Our results show that, in conjunction with elevated serum ALP level, oral exposure to pharmaceutical effluent at highest concentration (40 % v/v) elevated serum uric acid (SUA). This proposes further that, water contamination with pharmaceutical effluent may predispose an individual to cardiovascular diseases. Similarly, previous studies have reported increased SUA as an independent predictor of renal dysfunction in diseases such as rheumatoid arthritis and congestive heart failure (Daoussis et al., 2009; Tian et al., 2012). Therefore, the elevated SUA in our study may be a consequence of impaired renal function, which resulted to reduced excretion of UA. This thought is

supported further by concomitant increase in serum creatinine and electrolyte imbalance in our results (Figure 1a and Table 1).

Increase in serum creatinine in the exposed groups may be an indication of acute kidney injury, chronic kidney disease and renal dysfunction. Once creatinine is produced, it is immediately removed from the body by the kidney via urine. Since elimination of this molecule is solely by kidney, serum creatinine is widely used as a marker for renal function and readily employed in assessing acute kidney injury and chronic kidney disease (Winnett, et al., 2011; Baumgarten, 2011). Although, serum creatinine as a sole marker of kidney function has limitations; the observed electrolyte imbalance is a further confirmation of kidney dysfunction. The kidneys play a fundamental role in the regulation of body fluids and electrolytes; hence, impairment of kidney function results in derangement in electrolytes (Dhondup and Qian, 2017). Importantly, the observed hyperkalemia, hyperchloremia, hyponatraemia and elevated serum bicarbonate in the exposed animals have been earlier reported as predictors of chronic kidney disease and end-stage renal diseases (Dobre et al., 2015; Suetrong et al., 2016; Lim et al., 2016; Dhondup and Qian, 2017). Therefore, drinking water, contaminated with pharmaceutical effluent, may lead to development of chronic kidney disease and end-stage renal diseases. Interestingly, renal dysfunction has been noted to be a strong independent predictor of cardiovascular outcomes and mortality in the general population (Go et al., 2004), after myocardial infarction (Anavekar et al., 2004) and heart failure (Hillege et al., 2000). It can therefore be inferred from this study that, water contamination with pharmaceutical effluent may perhaps promote the pathogenesis of cardiovascular diseases via kidney function impairment.

The cardiorenal effects of pharmaceutical effluent observed in this study may be attributed to the combined effects of the chemical constituents. Chemicals such as Fe, Mn and NH_3 were found to be above permissible limit in our effluent sample. Meanwhile, iron overload has been reported to increase the fragility and density of renal lysosomes, thus, affecting their activities (Dimitriou et al., 2000). Since renal lysosomes play an important role in mediating kidney function of maintaining water-electrolyte homeostasis (Surendran et al., 2014), the disruption of serum electrolytes balance observed in this study may be due to renal iron accumulation. Similarly, cardiotoxicity and tissue damaging effect of Mn and NH_3, respectively, have been previously reported (Millera et al., 2006; McDaniel et al., 2016), hence, presence of these chemicals in the effluent probably resulted to impairment in cardiac enzymes activities.

The results of this study demonstrated that, drinking water, contaminated with pharmaceutical effluent, may disrupt enzyme activities, cause electrolytes imbalance and elevate serum uric acid. Therefore, such water may independently predispose individuals to cardiorenal syndrome. Further study, investigating the histology of concerned organs and mechanism(s) of cardiorenal toxicity of pharmaceutical effluent, should be carried out to exploit its roles in pathogenesis of kidney and cardiovascular diseases.

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