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Research article

Nephroprotective Effects of Exercise on Cisplatin Induced Acute Kidney Injury

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ABSTRACT

The relationship between exercise and renal function is a rather controversial topic. This study is aimed at investigating the overall effect of exercise as a preventive as well treatment option in the management of cisplatin-induced acute kidney injury (AKI). 25 male albino Wistar rats were acclimatized for two weeks then randomly divided into five groups (n=5): Group 1 (Control), Group 2 (Exercise group), Group 3 (cisplatin-induced), Group 4 (cisplatin +Exercise), Group 5 (Exercise+cisplatin). The animals in group 3, 4 and 5 were induced with AKI using intraperitoneal administration 3.0mg/kg Cisplatin which was administered for 3 days with a daily interval between each dose. The rats in group 2, 4 and 5 were subjected to aerobic exercise as a moderate regular exercise on a treadmill machine at the speed of 3km/h for 5 minutes at 0% inclination for 5 days/week for 3 week. After which the rats were sacrificed and blood, urine and kidney samples were obtained for laboratory analysis. Urine and serum electrolyte, creatinine and urea concentrations as well as urine albumin and serum cystatin C (Cys-C) concentration were analyzed. The finding of this study indicates that exercise when used as a treatment measure for cisplatin-induced AKI produced a mild functional and structural improvement. However, exercise as a preventive measure demonstrated a stronger tolerance to the toxicity of cisplatin structurally but not functionally. This study also showed cys-C was a poor biomarker for the detection of AKI in rat subjects.

Keywords: *Acute Kidney injury, cisplastin, exercise, cystatin-C*

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INTRODUCTION

Acute kidney injury (AKI) is defined as the sudden decline in kidney function generally demonstrated by either a rise in serum creatinine of at least 50% over baseline levels occurring within a 7-day time period, or a sudden decrease in urine output (KDIGO AKI Work Group, 2012; Asad *et al*., 2020). AKI disrupts the role of the kidneys in the maintenance of homeostasis in the body which ultimately has dire effect on the general physiology of the body systems (Jefferson *et al*., 2010). Therefore, early detection of AKI is crucial.In addition to creatinine there are numerous biomarkers for the determination of AKI, including neutrophil gelatinase-associated lipocalin (NGAL), N-acetyl beta D-glucosaminidase (NAG), kidney injury molecule-1 (KIM-1), interleukin-18, cystatin C, liver-type fatty acid binding protein, insulin-like growth factor binding protein 7 and tissue inhibitor metalloproteinase 2 (Joyce *et al*. 2020).

AKI has been seen to affect about 30–60% of critically ill patients and is associated with acute morbidity as well as mortality (Hoste, *et al*. 2018). A number of studies have estimated AKI to be responsible for approximately 2 million deaths annually worldwide (Ali *et al*., 2007; Murugan and Kellum, 2011; Chawla and Kimmel, 2012).

Exercise when performed regularly is known to have a host of physical and psychological benefits ultimately leading to the prevention and maintenance of various chronic diseases (Booth *et al*. 2012). However; when studying renal function in relation to exercise a worthy consideration is the effect exercise has on the renal blood flow (RBF). During exercise, increased sympathetic tone leads to increased cardiac output which serves to increase blood flow to the skeletal muscles to aid with the performance of exercise, increased core body temperature also increases blood flow to the skin, both of which may reduce RBF (Pechter *et al*. 2003; Swaminathan *et al*. 2018). Additionally, if exercise involves repeated eccentric muscle contractions, it can cause skeletal muscle damage, which may result in extracellular fluid entering the muscle cells (Miyagi *et al*. 2014; de-Lima *et al*. 2019). These effects may decrease plasma volume and activate the renin-angiotensin-aldosterone system (RAAS), which may also contribute to reduced RBF during exercise (de-Lima *et al*. 2019). Hypohydration is also known to decrease plasma volume (resulting in subsequent RAAS activation), increases circulating vasopressin, and increases core body temperature during exercise, all of which may further reduce RBF (Pechter *et al*. 2003; de Lima *et al*. 2019). During exercise, these factors

may combine to reduce RBF, which has been reported to decrease by as much as 75% during vigorous exercise (Swaminathan *et al*. 2018; Asad *et al*. 2020). A reduction in RBF may lead to renal ischemia and subsequent renal ATP depletion, which may also initiate AKI (Oliveira *et al*. 2017). Other school of thoughts believes that exercise can improve the kidney function. Inflammation within the kidneys after the initial insult to the kidney is a common occurrence known to increase and sustain the phases of renal injury which ultimately causes the scarring and permanent loss of part or the whole kidney (Friedewald and Rabb 2004). The exact mechanism in which exercise may protect against or even promote recovery following AKI in humans remains unclear; although one mechanism which may be crucial is the well-reported anti-inflammatory effect of exercise (Miyagi *et al*. 2014; Zeynali *et al*. 2015; Francescato *et al*. 2018; Asad *et al*., 2020).

Cancer is a disease with high prevalence. Cisplatin is considered as one of the most effective anticancer drugs used widely for the treatment of solid tumors (Aldossary, 2019). Despite its relevance in cancer treatment, cisplatin has been reported to have nephrotoxic effect thereby impairing kidney function (Dulz *et al*., 2017; Sarin *et al*., 2018).This study therefore aimed at investigating the overall effect of exercise in the prevention as well treatment option in the management of cisplatin-induced acute kidney injury.

MATERIALS AND METHODS

Experimental Animals: Twenty-five (25) male albino Wistar rats weighing between 225 – 290g were purchased from the Department of Anatomy, School of Basic Medical Science, University of Benin, Benin city, Edo state. The animals were housed in the animal house of anatomy department and acclimatized for two weeks with a standard environmental condition of 12 hours light and 12 hours dark cycle. The animals had adequate ventilation and were fed with standard rat chow and water ad libitum throughout the course of the study in accordance with the guidelines of National Research Council guide for care and use of laboratory animals as documented by (NRC, 1996)

Method of Induction: The animals were induced with AKI using intraperitoneal administration of 3.0mg/kg Cisplatin which was administered for 3 days with a daily interval between each dose.

Experimental Protocol: Following the acclimatization period, the animals were randomly divided into five groups (n=5 per group). Group 1 (control) was not induced or treated throughout the study. Group 2 (Exercised) was not induced but put through exercise treatment. Group 3 (Cis-induced) was induced but did not undergo exercise treatment. Group 4 (Cis-induced then exercised) was induced before they were put through the exercise treatment. Group 5 (Exercised then cis-induced) was put through exercise treatment before being induced. All groups received normal rat chow and drinking water throughout the study.

Exercise Protocol: The rats were subjected to aerobic exercise as a moderate regular exercise on a treadmill machine (Department of Physiology laboratory, University of Benin). After the acclimatization period animals in group 3 and 5 were subjected to treadmill exercise on the apparatus at the speed of 3km/h for 5 minutes for 5 days/week for 3 week, following the animals in group 5 were induced. The animals in group 4 were induced before receiving progressive exercise with a speed of 3km/h for 5 minutes for 5 days/week for 3 weeks. The angle of inclination will be at 0% during the entire study. Oxygen consumption for rats is said to be at about 65% at 0% inclination (Baranowski, *et al*. 2011; Noroozi *et al*. 2015; Zeynali, *et al*. 2015).

Collection of Samples

Urine Samples: Urine samples were collected over a 24-hour period using a metabolic cage.

Blood Samples: Animals were sacrificed after research period and blood was collected from the heart specifically the inferior vena cava through Cardiac Puncture. Sample was collected in a plain sample bottle and spun to obtain serum for testing.

Organ: The kidneys were isolated and fixed using formal saline, then sent for histological analysis.

Biochemical Analysis: Urine and serum electrolyte concentration were determined used an Ion Selective Electrode (ISE 4000) (Ahmad and Ahmad 2003).Serum and urine creatinine levels were measure using the colorimetric method (Bonsnes and Taussky 1945).Urea concentration in the urine and serum were determined using enzymatic method (Zawada, *et al*. 2009).Urine albumin levels were measured using a spectrophotometry analysis (Domon and Aebersold 2006).Serum cystatin C concentration was determined using the ELISA method (Engvall and Perlmann 1971)

Statistical Analysis: The Graph pad prism statistical software version 8.0.1 was used for data analysis. Comparison within groups was done using one-way ANOVA and comparison between groups was done using the Duncan's Multiple Range test.The results were presented as Mean ±SEM (Standard Error of Mean) and P-value of less than 0.05 (<0.05) was considered statistically significant and P-value greater than 0.05 (>0.05) was considered insignificant.

RESULTS

Effect on Electrolyte: Urine Electrolyte (Sodium, Chloride, Potassium and Bicarbonate) and the Serum Potassium concentrations showed no significant difference across all the groups as seen in Figure 1B, 2B, 3B, 4A and 4B. However, the serum concentration of Sodium, Chloride and Potassium showed significant decrease in the Cis-Induced (not treated) and the Exercised then Cis-induced groups when compared to the Exercised only group (Figure 1A, 2A, 3A).

The Exercised then Cis-induced group also showed significant decrease in the serum concentration of Sodium, chloride and potassium ions when compared to the Cis-induced then exercised group (Figure 1A, 2A, 3A). Serum concentration of sodium and chloride ions also showed a

significant decrease in the Cis-induced (not treated) and the Exercised then cis-induced groups when compared to the control group (Figure 1A, 2A).

Effect on Urea: Serum Urea concentration and BUN showed significant increase in the Cis-induced (not treated), Cis-induced then exercised and the Exercised the cis-induced groups when compared to the control and exercised only groups (Figure 5A, 7B). Cis-induced (not treated) group showed a significant increase in urine urea concentration when compared to the control group and the Cis-induced then exercised group showed significant decrease when compared to the Cis-induced (not treated) group (Figure 5B)

Effect on Creatinine: Serum creatinine concentration showed significant increase in the Cis-induced (not treated) and exercised then cis-induced groups when compared to the control and exercised only groups (Figure 6A). The Cis-induced then exercised showed significant increase in the urine creatinine concentration when compared to the control, exercised only and cis-induced (not treated) groups (Figure 6B)

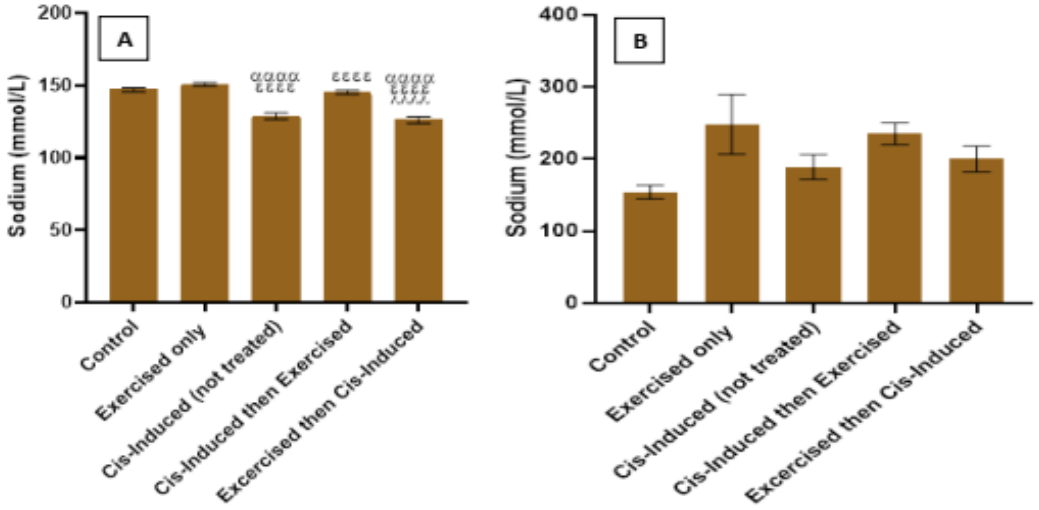

Figure 1:
(A) Serum sodium concentration result showing statistically significant difference among the different groups. (B) serum sodium concentration showing no statistically significant difference among the groups
[a]p<0.05 as compared with the control; [ε]p<0.05 as compared with the exercised only group ; [δ]p<0.05 as compared with the cisplatin induced group; [λ]p<0.05 as compared with the cisplatin induced then exercised group

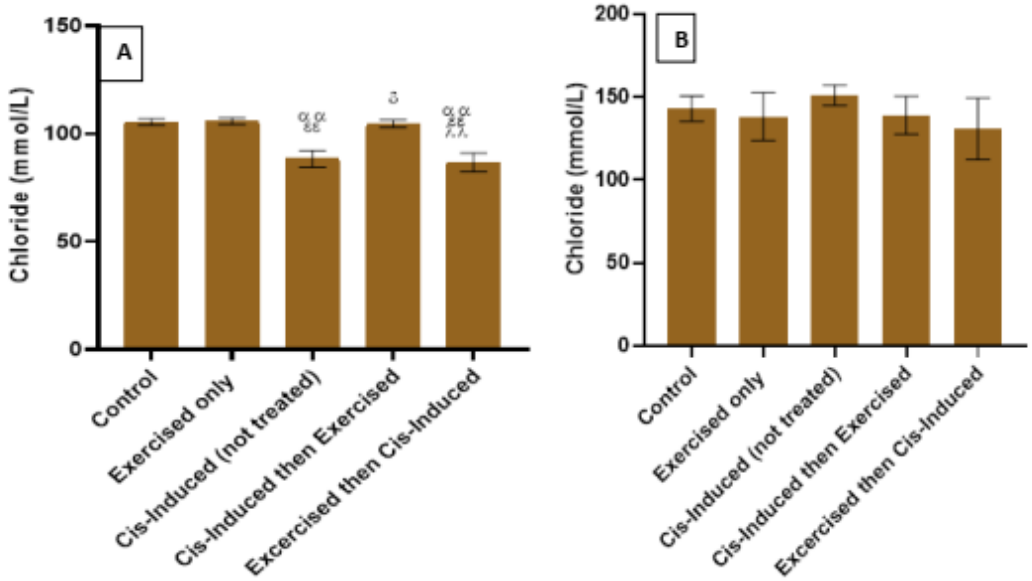

Figure 2:
(A) Serum chloride concentration result showing statistically significant difference among the different groups. (B) serum chloride concentration showing no statistically significant difference among the groups
[a]p<0.05 as compared with the control; [ε]p<0.05 as compared with the exercised only group ; [δ]p<0.05 as compared with the cisplatin induced group; [λ]p<0.05 as compared with the cisplatin induced then exercised group

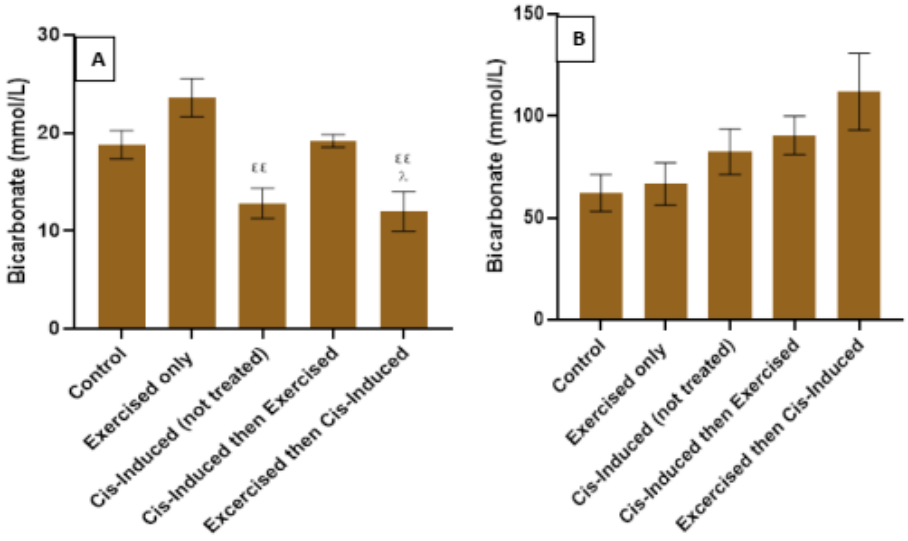

Figure 3:
(A) Serum bicarbonate concentration result showing statistically significant difference among the different groups. (B) Serum bicarbonate concentration showing no statistically significant difference among the groups
$^{\varepsilon}p<0.05$ as compared with the exercised only group ; $^{\delta}p<0.05$ as compared with the cisplatin induced group;
$^{\lambda}p<0.05$ as compared with the cisplatin induced then exercised group

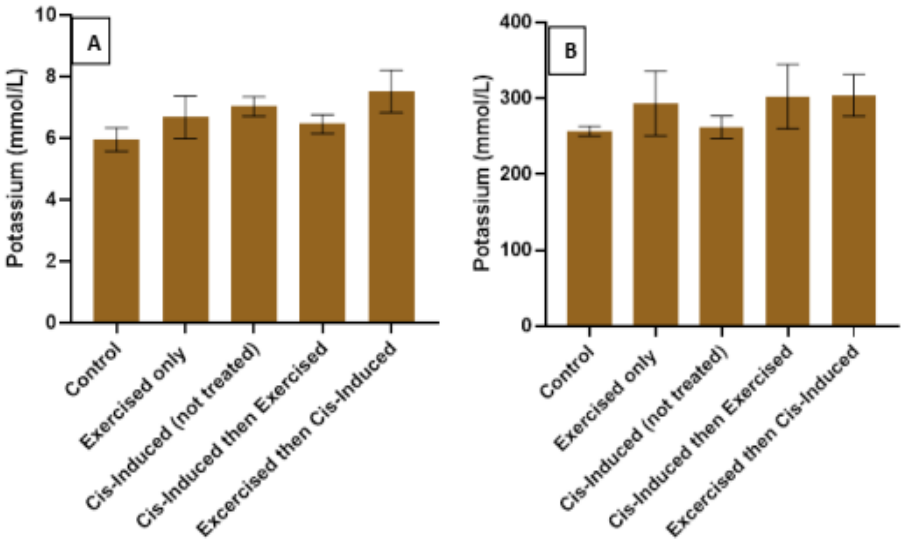

Figure 4:
(A) Serum potassium concentration result showing no statistically significant difference among the different groups. (B) serum potassium concentration showing no statistically significant difference among the groups
$^{a}p<0.05$ as compared with the control; $^{\varepsilon}p<0.05$ as compared with the exercised only group ; $^{\delta}p<0.05$ as compared with the cisplatin induced group; $^{\lambda}p<0.05$ as compared with the cisplatin induced then exercised group.

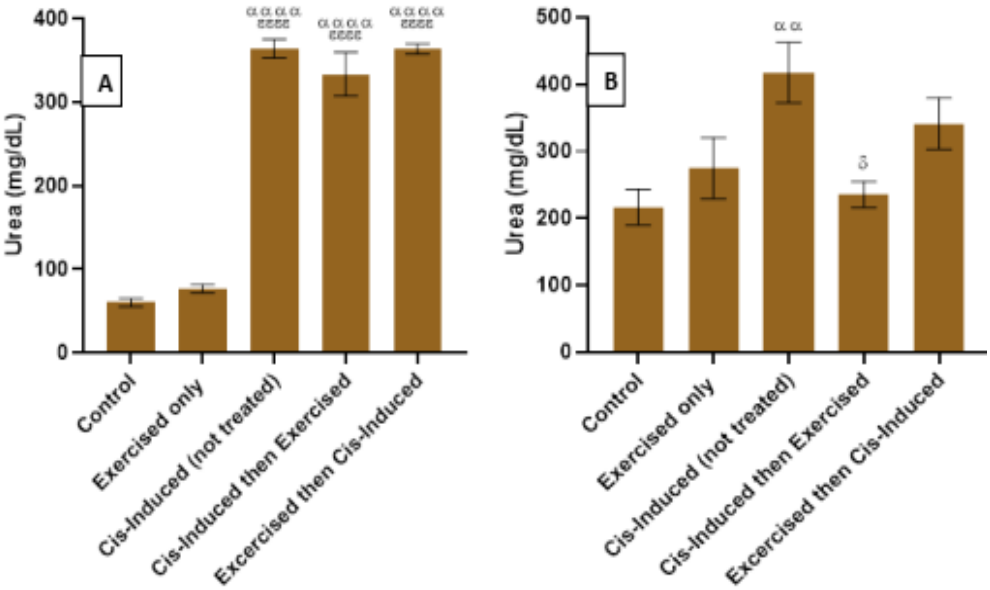

Figure 5:
(A) Serum urea concentration result showing statistically significant difference among the different groups. (B) serum urea concentration showing statistically significant difference among the groups.
$^{a}p<0.05$ as compared with the control; $^{\varepsilon}p<0.05$ as compared with the exercised only group ; $^{\delta}p<0.05$ as compared with the cisplatin induced group; $^{\lambda}p<0.05$ as compared with the cisplatin induced then exercised group.

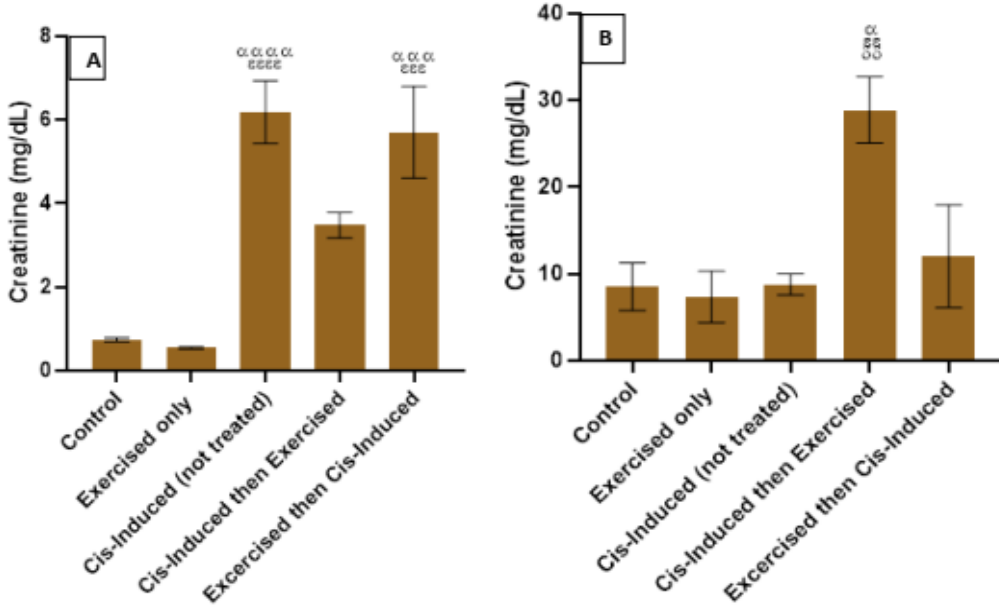

Figure 6:
(A) Serum creatinine concentration result showing statistically significant difference among the different groups. (B) serumcreatinine concentration showing statistically significant difference among the groups.
[a]p<0.05 as compared with the control; [c]p<0.05 as compared with the exercised only group ; [d]p<0.05 as compared with the cisplatin induced group; [f]p<0.05 as compared with the cisplatin induced then exercised group.

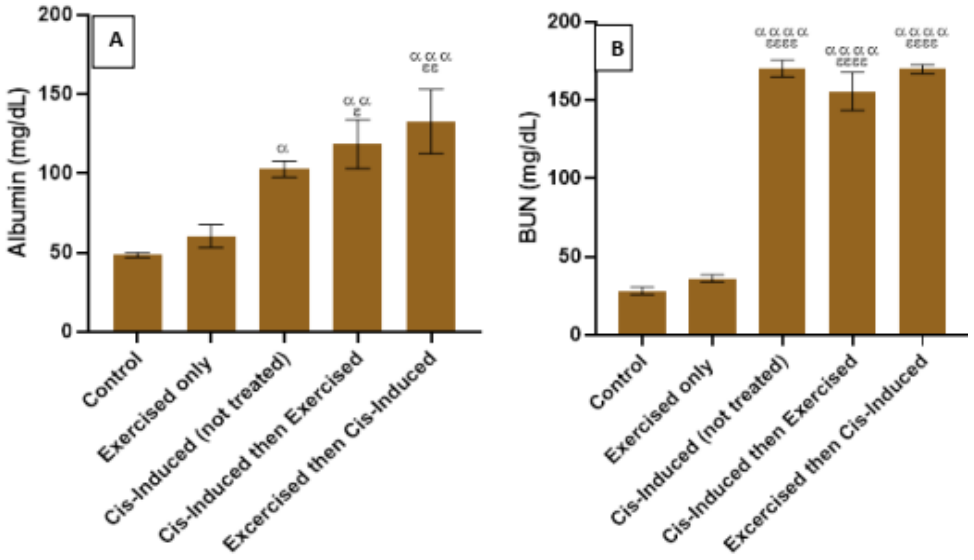

Figure 7:
(A) Urine albumin concentration result showing statistically significant difference among the different groups. (B) BUN concentration showing statistically significant difference among the groups.
[a]p<0.05 as compared with the control; [c]p<0.05 as compared with the exercised only group ; [d]p<0.05 as compared with the cisplatin induced group; [f]p<0.05 as compared with the cisplatin induced then exercised group

Effect on Albumin: There was a significant increase in the Albumin levels in the Cis-induced (not treated), Cis-induced then exercised and exercised the cis-induced when compared to the control. A Significant increase was also seen in the Cis-induced the exercised and exercised then cis-induced groups when compared to the exercised only groups (Figure 7A).

Effect on Cystatin C: There was no significant difference in cystatin C concentration across all groups (Figure 8).

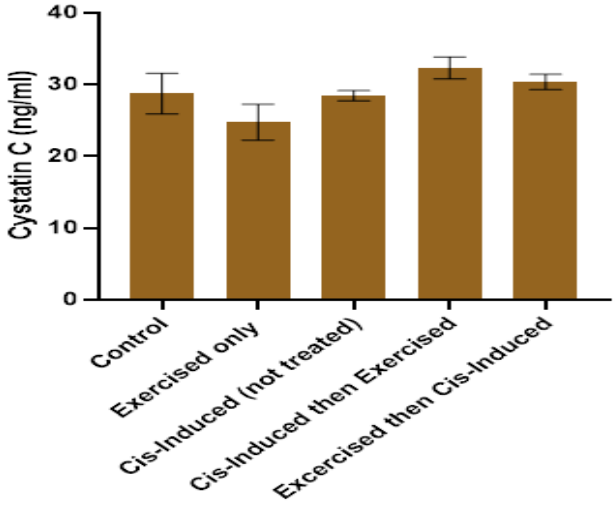

Figure 8:
Serum cystatin C concentration result showing statistically significant difference among the different groups

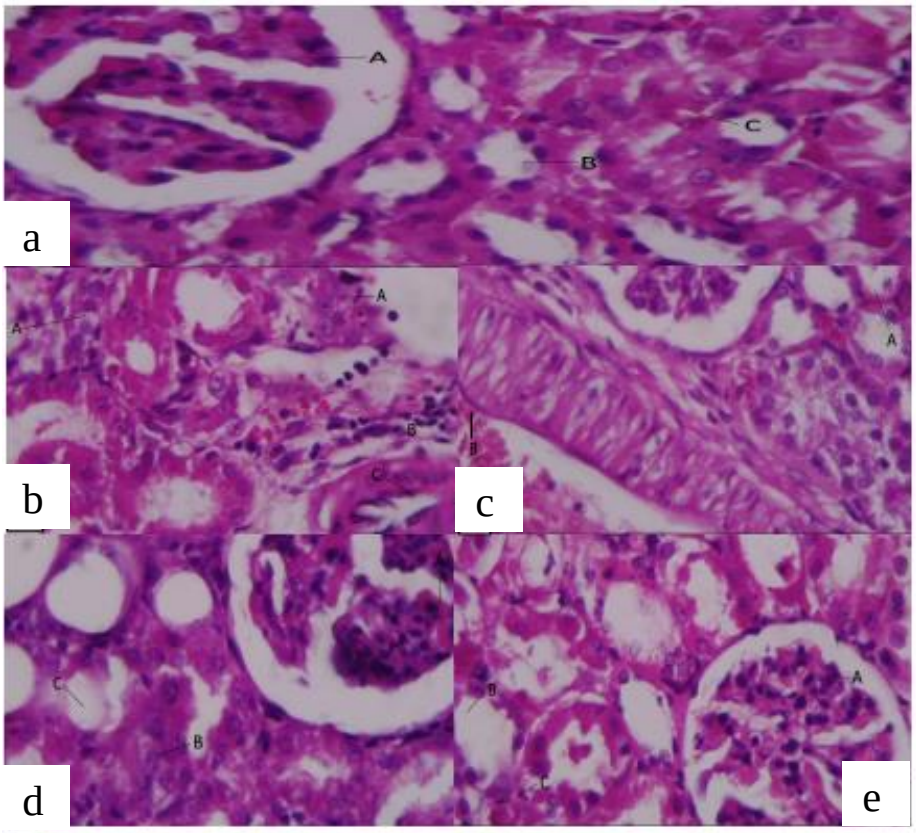

Plate 1:
Kidney histology plates stained with H&E (400). (a) Control (b) Cis-induced (c) Exercised (d) Cis-induced then exercised (e) Exercised then cis-induced.

Plate 1a: shows normal architecture with glomerulus (A), Tubules (B) and Interstitial space (C).
Plate 1b: Shows focal tubular necrosis (A), Interstitial infiltrates of inflammatory cells (B), and Vascular stenosis (C).
Plate 1c: Shows tubules with patent lumen (A) and active vascular congestion and dilatation (B)
Plate 1d: Shows normal glomerulus (A), focal tubular necrosis (B) and normal tubules (C)
Plate 1e: Shows normal architecture with glomerulus (A), tubules (B) and

DISCUSSION

The nephrotoxic side effect of cisplatin treatment is well noted, as the kidney plays an important role as the main route of cisplatin excretion (Dulz *et al*., 2017). Previous studies have suggested that the kidney has a tendency to accumulate cisplatin to higher levels compared to any other organ in the body including the liver (Dulz *et al*., 2017; Sarin *et al*., 2018). An adequately functional renal system is very essential for normal systemic body function so the presence of AKI essentially leads to a host of physiological problem. In this study cisplatin which is a chemotherapy drug known for its nephrotoxic qualities was used to induce AKI in animal subjects. Exercise was administered to evaluate its effect on the renal function as well as in alleviating the toxic effect of cisplatin.

The group which was treated with exercise but not subjected to AKI induction when examined histologically showed increased blood flow (active congestion), vasodilatation and increased the patency of the tubular lumen (opening up) and generally retaining the normal architecture of the nephron. This group showed a fairly normal functional rate when compared to the control with the levels of distinguishing biomarkers like creatinine, albumin, urea as well as electrolytes (Na+, K+, Cl- and HCO3-) being within the normal ranges.

In the cis-induced group there was an evident decrease in renal function when compared with the control group. When examined histologically there was focal tubular necrosis, interstitial infiltrates of inflammatory cells as well as vascular stenosis. Serum creatinine levels showed a statistically significant increase when compared with the control group which is in line with the definition of AKI as the sudden decline in kidney function demonstrated by either a rise in serum creatinine of at least 50% over baseline levels occurring within a 7-day time period, or a sudden decrease in urine output (Asad *et al*., 2020), The rise in serum creatinine as a biomarker for AKI detection has been supported by a number of studies (Lassnigg *et al*., 2004; Chertow *et al*., 2005; Kellum *et al*., 2021). This group also showed increased serum urea and BUN levels, a result supported by (Traynor *et al*. 2006). There was also a significant increase in urinary urea levels. Serum Na+, HCO3- and Cl- levels were seen to significantly decrease which indicate low reabsorption rate across the tubules, as previous studies have shown that proximal tubular damages at an early stage of toxicity results in reduction of reabsorption rate of water and electrolytes (Brozovic, *et al*., 2010; Dmitriev, 2011; Kim, *et al*., 2015). Albuminuria was also increased indicating glomerular damage which affects glomerular filtration allowing the increased secretion of protein, this is supported by (Dehne, et.al., 2010).

Exercise when administered as a treatment measure after the induction of AKI showed a mild improvement in the renal architecture with tubular necrosis still evident. In this group there was a significant increase of the serum Na+, HCO3- and Cl- levels as well as urinary urea levels when compared to the cis-induced group indicated tubular repair enabling increased reabsorption across the tubules. Serum creatinine levels in this group were seen to decrease compared to that of the cis-

induced group. However, there was a great increase in the urinary creatinine levels which could be as a result of muscle wasting during exercise supported by Hessels *et al*. in 2018 that reported urinary creatinine excretion to be normally equal to creatinine production, irrespective of the serum creatinine concentration (Hessels *et al*. 2018). The high level of creatinine in the urine as compared to the level present in the serum indicates glomerular repair and the effective filtration of creatinine by the tubules. There was however no improvement in albuminuria levels when compared to the cis-induced group.

When exercise was administered as a preventive measure before the induction of AKI, there was no potent effect of the nephrotoxicity of cisplatin as there was a fairly normal renal architecture when viewed histologically compared to the cis-induced group, this is supported by a 2018 study carried out by Francescato and his colleagues which showed that exercise training before cisplatin injection reduced renal damage induced by this drug evident as reduction of inflammatory response and amelioration of characteristic endothelial lesions (Francescato *et al*. 2018). Conversely, when functionally compared to the cis-induced group there was no significant improvement in the serum Na+ and Cl- levels, as well as the creatinine levels and albuminuria.

Serum cystatin C has been postulated by a number of studies as a potent early biomarker over creatinine (Murty et.al,. 2013; Leem *et al*., 2017; Afolayan *et al*., 2020). However, in this study there was no significant difference in serum cystatin C levels across all groups despite the notably statistically significant rise in serum creatinine. This suggests cystatin C to be a poor marker for the detection of AKI which is in line with a study by Hamed and colleagues in 2013 where they showed serum cystatin C as a poor biomarker for detection of AKI in critically-ill children (Hamed *et al*., 2013). The inadequacy of cystatin C in the detection of AKI has been reported by a number of studies (Ristikankare *et al*., 2010; Bongiovanni *et al*., 2015; Safdar *et al*., 2016).

In conclusion, the findings of this study indicates that exercise when used as a treatment measure for cisplatin-induced AKI produced a mild functional and structural improvement and should be further explored. However, exercise as a preventive measure demonstrated ameliorative effect to the toxicity of cisplatin structurally but did not show any notable improvement functionally. Therefore, the combination of exercise together both as a preventive and treatment option might pose a better plan for the management of cisplatin-induced (nephrotoxic) AKI. Further, from this study cystatin C was seen as a poor biomarker for the detection of AKI in rat subjects.

Contribution to Knowledge

Due to the necessity of cisplatin as a chemotherapy treatment in cancer patients, its usage is quite a necessity. Patients who are to undergo cisplatin treatment are advised to partake in moderate exercise to help ameliorate the nephrotoxic effect of the drugs and ensure a more beneficial outcome of the treatment plan.

REFERENCES

Afolayan, F.M., Adedoyin, O.T., Abdulkadir, M.B., Ibrahim, O.R., Biliaminu, S.A., Mokuolu, O.A.,&Ojuawo, A. (2020). Acute Kidney Injuries in Children with Severe Malaria A comparative study of diagnostic criteria based on serum cystatin C and creatinine levels. *SQU Med J*; 20(4): 312–317.

Ahmad, G.R.,& Ahmad, D.R. (2003). Electrolytes analysis.Ency.*Food Sci. Nutr*.;2:2036-2038

Aldossary, S.A. (2019). Review on Pharmacology of Cisplatin: Clinical Use, Toxicity and Mechanism of Resistance of Cisplatin. *Biomed.&Pharmacol. J*;12(1): 7-15.

Ali, T., Khan, I., Simpson, W., Prescott, G., Townend, J., Smith, W., & Macleod, A. (2007). Incidence and outcomes in acute kidney injury: a comprehensive population-based *study.J Am SocNephrol*; 18: 1292–1298.

Asad, A., Burton, J.O.,& March, D.S. (2020). Exercise as a therapeutic option for acute kidney injury: mechanisms and considerations for the design of future clinical studies. *BMC Nephrol*; 21:446.

Baranowski, M., Zabielski, P., Blachnio-Zabielska, A.U., Harasiuk, D., &Gorski, J. (2011). LXR activation prevents exhaustive exercise-induced hypoglycemia and spares muscle glycogen but does not en¬hance running endurance in untrained rats. *ActaPhysiol (Oxf);* 201(3):373–379.

Bongiovanni, C., Magrini, L., Salerno, G., Gori, C.S., Cardelli, P., Hur, M., Buggi, M., &Somma, S.D. (2015). Serum Cystatin C for the Diagnosis of Acute Kidney Injury in Patients Admitted in the Emergency Department. Disease Markers; Article ID 416059: 1-7.

Bonsnes, R.W., &Taussky, H.H. (1945). On the colorimetric determination of creatinine by the JAFFE reaction. *J. BioChem*;158(3): 581-591

Booth, F.W., Roberts, C.K., &Laye, M.J. (2012). Lack of exercise is a major cause of chronic diseases. *ComprPhysiol*; 2:1143-1211.

Brozovic, A., Ambriovic-Ristov, A., &Osmak, M. (2010). The relationship between cisplatin-induced reactive oxygen species, glutathione, and BCL-2 and resistance to cisplatin. *Crit Rev Toxicol*; 40(4): 347-359.

Chawla, L.S., &Kimmel, P.L. (2012). Acute kidney injury and chronic kidney disease: an integrated clinical syndrome. *Kidney Int.*; 82: 516–524.

Chertow, G.M., Burdick, E., Honour, M., Bonventre, J.V., &Bates, D.W. (2005). Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. *J. Am. Soc. Nephrol*. 16, 3365–3370.

De Lima, W.V., Visona, I., Schor, N., &Almeida, W.S. (2019). Preconditioning by aerobic exercise reduces acute ischemic renal injury in rats. Physiol Rep; 7(14): e14176.

Dehne, M.G., Hartmann, B., Katzer, C., &Röhrig, R. (2010). Early diagnosis of acute kidney injury. *Cent. Eur. J. Med*.5(5) 527-534

Dmitriev, O.Y. (2011). Mechanism of tumor resistance to cisplatin mediated by the copper transporter ATP7B.*Biochem Cell Biol*; 89(2): 138-147.

Domon, B., &Aebersold, R. (2006). Mass Spectrometry and Protein Analysis.*Sci*.; 312: 212-217

Dulz, S., Asselborn, N.H., Dieckmann, K.P., Matthies, C., Wagner, W., Weidmann, J., Seidel, C., Oing, C., Berger, L.A., Alsdorf, W., Mankichian, B., Meyer, C., Vetterlein, M.W., Gild, P., & Ludwig, T.A. (2017). Retinal toxicity after cisplatin-based chemotherapy in patients with germ cell cancer.*J Cancer Res ClinOncol*; 143(7): 1319-1325.

Engvall, E., &Perlmann, P. (1971). Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. *Immunochemistry*;8(9): 871-874

Francescato, H.D.C., Almeida, L.F., Reis, N.G., Faleiros, C.M., Papoti, M., Costa, R.S., &Coimbra, T.M. (2018). Previous Exercise Effects in Cisplatin-Induced Renal Lesions in Rats.Kidney *Blood Press Res*; 43:582-593.

Friedewald, J.J., &Rabb, H. (2004). Inflammatory cells in ischemic acute renal failure. *Kidney Int*; 66(2):486–491.

Hamed, H.M., El-Sherbini, S.A., Barakat, N.A., Farid, T.M., &Rasheed, E.A. (2013). Serum cystatin C is a poor biomarker for diagnosing acute kidney injury in critically-ill children. *Indian J Crit Care Med*; 17:92-98.

Hessels, L., Koopmans, N., Neto, A.W.G., Volbeda, M., Koeze, J., Lansink Hartgring, A.O., Bakker, S.J., Straaten, H.M.O., &Nijsten, M.W. (2018). Urinary creatinine excretion is related to short-term and long-term mortality in critically ill patients. *Intensive Care Med*;44:1699–1708

Hoste, E.A.J., Kellum, J.A., Selby, N.M., Zarbock, A., Palevsky, P.M., &Bagshaw, S.M.(2018). Global epidemiology and outcomes of acute kidney injury. *Nat Rev Nephrol*; 14(10):607–625

Jefferson, J.A., Thurman, J.M., &Schrier, R.W. (2010). Pathophysiology and etiology of acute kidney injury.*Com ClinNephro*l; 797-812.

Joyce, E. L., Kane- Gill, S. L., Priyanka, P.,Juett, L.A., James, L.J., &Mears, S.A. (2020). Effects of Exercise on Acute Kidney Injury Biomarkers and the Potential Influence of Fluid Intake.*Ann NutrMetab*; 76(1):53–59.

KDIGO AKI Work Group. (2012). KDIGO clinical practice guideline for acute kidney injury.Kidney Int. Suppl. 2, 1–138.

Kellum, J.A., Romagnani, P., Ashuntantang, G., Ronco, C., Zarbock, A., &Anders, H.J. (2021) Acute kidney injury. *Nat.Rev*.; 7:52.

Kim, H.J., Park, D.J., Kim, J.H., Jeong, E.Y., Jung, M.H., Kim, T.H., Yang, J.I., Lee, G.W., Chung, H.J., & Chang, SH. (2015). Glutamine protects against cisplatin-induced nephrotoxicity by decreasing cisplatin accumulation. *J PharmacolSci*; 127(1): 117-126. ISSN 1347-8613.

Lassnigg, A., Schmidlin, D., Mouhieddine, M., Bachmann, L.M., Drumi, W., Bauer, P., &Hiesmayr, M. (2004). Minimal changes of serum creatinine predict prognosis in patients after cardiothoracic surgery: a prospective cohort study.*J. Am. Soc. Nephrol*. 15(6), 1597–1605.

Leem, A.Y., Park, M.S., Park, B.H., Jung, W.J., Chung, K.S., Kim, S.Y., Kim, E.Y., Jung, J.Y., Kang, Y.A., Kim, Y.S., Kim, S.K., Chang, J., &Song, J.H. (2017). Value of Serum Cystatin C Measurement in the Diagnosis of Sepsis-Induced Kidney Injury and Prediction of Renal Function Recovery.Yonsei *Med J*; 58(3):604-612

Miyagi, M.Y., Seelaender, M., Castoldi, A., de Almeida, D.C., Bacurau, A.V., &Andrade-Oliveira, V. (2014). Long-term aerobic exercise protects against cisplatin-induced nephrotoxicity by modulating the expression of IL-6 and HO-1. *PLoS One;* 9(10): e108543

Murty, M., Sharma, U.K., Pandey, V.B., &Kankare, S.B. (2013) Serum cystatin C as a marker of renal function in detection of early acute kidney injury.*Indian J Nephrol*; 23(3):180-183.

Murugan, R., &Kellum J.A. (2011). Acute kidney injury: what's the prognosis? *Nat Rev Nephrol*; 7: 209–217.

National Research Council (NRC).(1996). National science education standards. No. National Academy Press. Washington: D.C.

Noroozi, J., Zeynali, F., Nematbakhsh, M., Pezeshki, Z., &Talebi, A. (2015). Nonpreventive role of aerobic exercise against cisplatin-induced nephrotoxicity in female rats.*Int J Prev Med*; 6:58.

Oliveira, C.S., Rodrigues, A.M., Nogueira, G.B., Nascimento, M.A., Punaro, G.R., &Higa, E.M. (2017). Moderate aerobic exercise on the recovery phase of gentamicin-induced acute kidney injury in rats.*Life Sci*; 169:37–42.

Pechter, U., Ots, M., Mesikepp, S., Zilmer, K., Kullissaar, T., &Vihalemm, T.(2003). Beneficial effects of water-based exercise in patients with chronic kidney disease. *Int J Rehabil Res*; 26(2):153–156.

Ristikankare, A. Pöyhiä, R., Kuitunen, A., Skrifvars, M., Hämmäinen, P., Salmenperä, M., &Suojaranta-Ylinen, R. (2010). Serum Cystatin C in Elderly Cardiac Surgery Patients.*Ann ThoracSurg*; 89:689 –695

Safdar, O.Y., Shalaby, M., Khathlan, N., Elattal, B., Joubah, M.B., Bukahri, E., Saber, M., Alahadal, A., Aljariry, H., Gasim, S., Hadadi, A., Alqahtani, A., Awleyakhan, R., &Kari, J.A. (2016). Serum cystatin is a useful marker for the diagnosis of acute kidney injury in critically ill children: prospective cohort study. *BMC Nephrol*; 17(130): 1-8.

Sarin, N., Engel, F., Rothweiler, F., Cinatl, J., Michaelis, M., Frohlich, H., &Kalayda, G.V. (2018). Key Players of Cisplatin Resistance: Towards a Systems Pharmacology Approach. *Int J MolSci*; 19(3).ISSN 1422-0067.

Swaminathan, M., Stafford-Smith, M., Chertow, G.M., Warnock, D.G., Paragamian, V., &Brenner, R.M. (2018). Allogeneic Mesenchymal stem cells for treatment of AKI after cardiac surgery.*J Am SocNephrol*; 29(1):260–267.

Traynor, J., Mactier, R., Geddes, C.C., &Fox, J.G. (2006). How to measure renal function in clinical practice. *BMJ*333:733

Zawada, R.J.X., Kwan, P., Olszewski, K.L., Llinas, M., &Huang, S.G. (2009). Quantitative determination of urea concentrations in cell culture medium.*Biochem Cell Biol*; 87(3): 541–544

Zeynali, F., Nematbakhsh, M., Mojtahedi, H., Poorshahnazari, A., Talebi, A., Pezeshki, Z., Mazaheri, S., &Moslemi, F. (2015). Protective Role of Aerobic Exercise AgainstCisplatin-Induced Nephrotoxicity in Rats. *Asian J Sports Med*; 6(3): e24901