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Effects of *Moringa oleifera* Tea Supplement on the Biochemical Indices of Diabetes and Hypertension Co-Morbidity Patients

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Abstract
Type 2 diabetes (T2D) and Hypertension are the most common co-morbidities which has received attention worldwide. This research investigates the effect of Moringa oleifera tea supplements (MOTs) on the biochemical parameters of diabetic and hypertensive co-morbidity patients. Twenty-five participants of both genders were randomly distributed into five groups containing five patients each; group 1 (positive control), group 2 (negative control), group 3 (positive control+MOT), group 4 (standard drug) and group 5 (standard drug+MOT). All treatments were carried out for 14 days after which blood samples were collected for biochemical analyses. The lipid biochemical profile, liver function parameters and renal function parameters of the patients were analysed using standard procedures. The study revealed that the consumption of MOTs significantly ($p<0.01$) increased the serum high-density lipoproteins and reduced low-density lipoproteins, triglycerides, and cholesterol concentrations of the patients that were given MOT supplements in comparison to the control patients. Conversely, there was significant ($p<0.01$) reduction in the activities of serum alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, and the concentration of albumin, total protein, direct bilirubin and total bilirubin in comparison to the negative control group. Hence, this study outcome revealed that supplementing the diet of diabetes and hypertensive patients' diet with MOTs is beneficial to the health of the patients and is equally safe for consumption.
Keywords: *Diabetes; hypertension; co-morbidity; Moringa oleifera; Tea supplements.*

INTRODUCTION

Diabetes and hypertension are the leading cause of disease worldwide, with the population suffering from these diseases steadily increasing globally despite being preventable (Al-Hajj *et al.,* 2021). According to the IDF report, there is an average of 451 million people between the age of 18 to 99 years suffering from diabetes in 2017 globally with the figures projected to rise to 693 million people by the year 2045 (Ogurtsova *et al*., 2017). Globally, approximately 1 billion people are reported to be hypertensive, and this population is expected to increase to 1.56 billion by 2025 globally (Tripathy *et al.,* 2017). Hypertension is the most prevalent co-morbidity condition in people suffering from diabetes (Tripathy *et al.,* 2017). Its prevalence depends on several factors such as sex, age, underlining aliment, type and duration of diabetes, (De Ferranti *et al.,* 2014). Studies have shown that patients suffering from diabetes have 2.5 times of suffering from hypertension than disease conditions (Ian *et al.,* 2017; Petrie *et al.,* 2018). The prevalence of these diseases continues to increase globally as there is a spontaneous increase in low energy expenditure and high caloric intake lifestyles adopted worldwide, especially in developing countries. Attention has now been drawn to the dietary management approach to these diseases (Petrie *et al.,* 2018).

Moringa oleifera is a family of *moringaceae* of Indian origin, widely grown and cultivated globally especially in Africa because of its potential health and nutritional benefit (Mgbemena and Obodo 2016). It is a drought-resistant plant and can survive with little supply of water (Mgbemena and Obodo 2016). MOT supplement possesses important bioactive compounds with important health benefits against chronic degenerative diseases (Lambe and Bewaji, 2017) such as diabetes and hypertension (Lako *et al.,* 2007; Oluduro, 2012). Several previous studies have reported the health benefits of the *Moringa oleifera* plant (Jaiswal *et al.,* 2013; Laleye *et al.,* 2017). Currently, there is a paucity of available information on the effect of MOT supplements on the biochemical indices of diabetes and hypertension co-morbidity patients.

Available information has shown that MOT supplement is commonly consumed, accepted, and steadily gaining favourites among people due to the easy availability, and nutritional and health benefit attributed to its raw

material (Asben and Rini, 2019). Studies have shown that the raw components (*Moringa Oleifera*) plant possesses antioxidant, antimicrobial, antiatherogenic, antitumoral, cytoprotective, antihypertensive, protective action for diabetes, obesity, neurodegenerative diseases, cardiovascular diseases and diabetes mellitus (Zhang *et al.,* 2019; Vergara-Jimenez, 2017).

Tea, according to Philip, Boma and Yahaya (2020) is extensively frequently consumed globally. Currently, there is high demand and increasing consumption of herbal tea globally due to its associated high nutritious value and health benefits (Xiao *et al.,* 2020). The flavour of herbal tea is derived from a blend of herbs, spices, botanicals, and natural flavours. Hence, this study was done to examine the effect of the consumption of MOT supplements on the biochemical indices of diabetes and hypertension co-morbidity patients.

MATERIALS AND METHODS

Materials

Moringa oleifera tea (MOT) (product of UvaHalpewatte Estate Limited, Sri Lanka) was purchased from Matrite shopping mall Ilorin Kwara State, Nigeria. Kits for lipid profile biochemical were products of Agape Diagnostics Switzerland GmbH. All reagents and chemicals used in this study were of analytical grade.

Experimental Design

The research was carried out at the Adewole Cottage Hospital Ilorin, Kwara State (Nigeria) in April 2021. Twenty-five (25) Type 2 diabetes (T2D) and hypertensive patients, aged between 35 and 65 years were engaged in this study. Patients under specific treatment monitoring (Tabs Amlodipine 5 mg daily and Tabs Metformin 1 g morning and night) scheme with the Doctors in the Cottage Hospital were engaged in this study and their consent was sorted before administration. *Moringa oleifera* tea bag per serving contains of 2g of *Moringa oleifera*. Patients were placed on a tea bag morning and night. Patients of either sex were randomised into five groups containing five Participants. Group-I (Positive Control) were patients with no ailment and no treatment. Group II were (Negative control) diagnosed patients without treatment. Group-III (Positive Control + MOT) patients with no ailment but were taking MOT supplements. Group-IV (Reference drug) patients were diagnosed with co-morbidity and under management with reference drug (Tabs Metformin and Amlodipine) Group-V (reference drug + MOT) patients diagnosed with co-morbidity and under management and were given MOT. All administrations were done for fourteen days, thereafter blood samples were collected and biochemical parameters were evaluated.

Sample Collection

After the expiration of 14 days, blood samples were collected by Phlebotomist. The sample was withdrawn into a clean, plain and sterile sample bottle, and then centrifuged at 3000 rpm for 15 minutes in a microcentrifuge to obtain clear serum. The samples were kept at 20˚C temperature until analyses were done.

Ethical Statement

The study design and ethical clearance were approved and granted by the Ethical Committee of Kwara State Ministry of Health, Ilorin, Nigeria, in consonance with the institutional guidelines. An approval number MOH/KS/EU/777/490 was assigned. All the participants gave their informed consent before the commencement of the study.

Determination of Lipid Profiles

The lipid assays were done using standard laboratory procedures. The serum HDL was estimated according to the combined enzymatic and phosphotungstate precipitation method described by Burstein *et al.* (1970) and Lopez-s *et al.* (1974). Briefly, a portion of 0.2 ml of the serum was added into tubes and 0.5 ml of reagent (A) was added in each of the tubes, mixed well and allowed to stand for 10 minutes. Thereafter, it was centrifuged for 10 minutes at 4000 rpm after which the supernatant was carefully decanted. Approximately 50 µl of the supernatant, distilled water and HDL-cholesterol standard were added each to separate tubes and 1.0 ml of reagent (B) pipetted into each of the tubes. The tubes were thoroughly mixed and incubated for 10 minutes at 37^{o}C. The absorbance of the standard and sample were measured at 500 nm wavelength against the blank. The LDL of the sample was estimated according to the combined polyvinyl sulphate precipitation and enzymatic procedure described by Assmann *et al*. (1984) and Greten *et al.* (1974). Briefly, 0.2 ml of serum was into tubes containing 0.2 ml of reagent (A). The mixture was vortexed and allowed to stand for 15 minutes at room temperature. Then, it was centrifuged for 15 minutes at 4000 rpm and the supernatant was then carefully decanted. Thereafter, approximately 20 µl of the supernatant, distilled water and cholesterol standard were added each into separate tubes and 1.0 ml of reagent (A) was added into each of the tubes, vortexed thoroughly and incubated for 10 minutes at 37^{o}C. Thereafter, the absorbance of the standard and sample were measured at 500 nm against the blank and the level of the LDL was computed. The level of Triglycerides was estimated according to enzymatic method described by Castelli *et al.* (1977). Briefly, approximately 10 µl of serum, distilled water and triglyceride standard were added each

in a separate tubes after which 1000 µl of reagent was added to each of the tubes, vortexed and incubated at 37°C for 5 minutes. Thereafter, the sample absorbance and absorbance of the standard was measured against the reagent blank within 60 minutes at 500 nm wavelength and the concentration of the serum triglycerides was estimated. Total cholesterol was estimated according to the enzymatic method described by Seidel *et al.* (1983) and Kattermann *et al.* (1984). Briefly, approximately, 10 µl of distilled water, cholesterol standard and serum were added each into separate tubes and thereafter 1000 µl of reagent was added into each of the tubes, mixed and incubated for 5 minutes at 37°C. Thereafter, the sample absorbance and absorbance of the standard were measured against the reagent blank within 60 minutes at 500 nm wavelength and the concentration of the serum total cholesterol was estimated.

Liver Function Indices

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity were estimated according to the method reported by Reitman and Frankel (1957) with slight modifications. Serum alkaline phosphatase (ALP) activity was determined according to the method reported by the procedure reported by Rec (1972). The total serum protein concentration was determined according to Biuret colourimetric method reported by Tietz (1976). The albumin level of the samples was estimated according to the colorimetric method reported by Doumas *et al.* (1971)**.** The direct and indirect bilirubin was determined according to the calorimetric method described by Jendrasick and Grof (1938) and Sherlock (1951) with slight modification**.**

Kidney Function Indices

The creatinine level of the serum sample was determined according to the method reported by Butler (1975). Uric acid was estimated according to the method reported by Collin and Diehl (1959) and Morin and Proxy (1973). Urea level was measured according to the colorimetric method procedure described by Evans (1968). Sodium level was measured according to the procedure described by Trinder (1951). Serum chloride concentration level was determined according to the procedure described by Schoenfeld and Lewellan (1964).

STATISTICAL ANALYSIS

The results of five (5) replicate determinations were expressed as the mean ± standard error of the mean (SEM). One-way analysis of variance was used to determine statistical differences between different groups in which $p<0.01$ is considered statistically significant. All statistical analysis was done using SPSS software version 26.

RESULTS

Effects of MOT Supplement on Lipid Profile of Diabetic/Hypertension Co-morbidity Patients

There was a significant ($p<0.01$) increase in the serum concentration of HDL-cholesterol in the MOTs group when compared to those of both positive and negative controls which were significantly ($p<0.01$) reduced. Whereas, no significant ($p>0.01$) difference was observed in the concentration of serum LDL of both the positive control and MOTs group, whereas not negative control group and the reference drug were significantly ($p<0.01$) higher. The serum TG and cholesterol were significantly ($p<0.01$) increased in the serum of the negative control patients compared to the control, whereas the level was significantly reduced in groups on reference drugs with more significant ($p<0.01$) reduction in groups supplemented with MOTs. However, the parameters of the lipid profile of the positive control supplemented with MOT were not significantly ($p<0.01$) different from the control (Table 1).

Effects of MOT Supplement on Liver Function Parameters of Diabetic/Hypertension Co-morbidity Patients

The result of the liver function biochemical parameters of the participants are presented in Table 2. It was revealed that there was a significant ($p<0.01$) increase in the liver function parameters of the negative control patients to the control and other treatment groups. Whereas, generally significant ($p<0.01$) reduction was also observed in the serum activities of AST, ALT, ALP and the concentration of ALB, TP, DB and TB when compared to the negative control patients.

Effects Supplement on Renal Function Indices of Diabetic/Hypertension Co-morbidity Patients

No significant($p>0.01$) alteration was observed in the kidney function indices of all the groups that were given MOT supplements when compared with those of the control patients except in the groups administered only standard drugs which had significant ($p<0.01$) increase in creatinine, urea, uric acid, Na^+, K^+, CL^- and HCO_3^- (Table 3).

Table 1: Lipid profile of diabetic/hypertension co-morbidity patients following the administration of MOTs

Treatment	HDL(mmol/L)	LDL(mmol/L)	TG(mmol/L)	Cholesterol(mmol/L)
Positive Control	1.33±2.50[a]	3.0±0.60[a]	1.20±0.20[a]	3.5±0.25[a]
Negative control	1.11±2.22[b]	4.5±2.40[b]	2.6±0.51[b]	7.8±0.61[b]
Positive Control + MOT	1.35±2.44[a]	3.20±0.50[a]	1.0±0.18[a]	3.0±0.25[a]
Standard drug	1.55±2.42[c]	4.1±1.40[c]	2.45±052[c]	6.8±0.30[c]
Standard drug + MOT	1.65±2.24[c]	3.5±1.32[a]	2.05±0.30[d]	6.10±0.10[d]

Data are expressed as Mean ±SEM. Values having the same superscripts in the same column are not significantly different ($p \leq 0.01$). HDL; high-density lipoproteins, LDL; Low-density lipoproteins, TG; triglycerides

Table 2: Liver function indices of diabetes-hypertension co-morbidity patients following the administration of MOTs

Treatment	AST (IU/L)	ALT (IU/L)	ALP(IU/l)	ALB (g/dL)	TP (g/l)	DB (umol/l)	TB (g/l)
Positive Control	19.6± 0.93[a]	37.8± 1.47[a]	45.8± 1.16[a]	3.9±0.29[a]	76.4± 2.87a	0.33± 0.04[a]	66.2± 2.51[a]
Negative Control	20.2± 0.81[b]	36.7± 1.20[a]	48.1± 1.21[b]	4.0±0.52[a]	76.2± 2.60a	0.35± 0.50[a]	67.1± 2.60[a]
Positive Control + MO tea	18.2± 2.75[a]	36.0 ± 1.95[a]	38.6± 1.17[c]	4.1±0.31[a]	72.0 ± 4.42[b]	0.27± 0.03[b]	56.0± 2.82[b]
(Standard drug)	26.8± 2.48[b]	31.4± 3.33[b]	47.4± 2.80[ab]	5.2±0.13[b]	79.2± 1.69[ab]	0.35± 0.06[a]	53.0 ± 0.05[c]
(Standard drug + MO tea)	22.6± 2.12[c]	30.4± 1.81[b]	35.6± 3.39[c]	4.8±0.16[b]	73.6± 1.36[b]	0.22± 0.03[c]	53.1± 3.41c

Data are expressed as Mean ±SEM. Values having the same superscripts in the same column are not significantly different ($p \leq 0.01$). AST; Aspartate Amino Transferase, ALT; Alanine Amino Transferase, ALP; Alkaline Phosphatase, ALB; Albumin, TP; Total Protein, TB; Total Bilirubin and DB; Direct *Bilirubin,

Table 3: Renal function indices of diabetes-hypertension co-morbidity patients following the administration of MOTs

Treatment	Creatinine (µmol/L)	Urea (mmol/L)	Uric Acid (mg/dl)	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	HCO_3^- (mmol/L)
Positive Control	99.6± 10.20[a]	4.62± 0.22[a]	0.32± 0.21[a]	140.5± 5.00[a]	4.67± 0.19[a]	92.51± 3.59[a]	35.32± 2.13[a]
Negative Control	112.5± 10.10[b]	4.52± 0.21[a]	0.28± 0.40[a]	141.2± 5.10[a]	4.50± 0.20[a]	95.50± 4.0[b]	36.20± 2.50[a]
Positive Control + MO tea	95.5± 10.70[a]	4.31± 0.21[b]	0.30± 0.20[a]	138.5± 2.5[a]	4.54 ± 0.36[a]	96.16± 4.06[b]	35.81± 0.87[a]
(Standard drug)	120.0 ± 10.60[b]	6.94± 0.33[c]	0.40± 0.15[b]	156.23±3.21[b]	5.48± 0.22[b]	100.43± 4.62[c]	42.76± 1.10[b]
(Standard drug + MO tea)	100.0 ± 10.50[a]	5.93± 0.26[c]	0.35± 0.15[b]	146.53±3.27[c]	5.38± 0.19[b]	98.29± 1.93[c]	38.94± 1.26[c]

Data are expressed as Mean ±SEM. Values having the same superscripts in the same column are not significantly different ($p \leq 0.01$)

DISCUSSION

Dyslipidemia is a common metabolic syndrome of diabetes and hypertension patients, this may be attributed to the insulin deficiency and resistance characterising the two metabolic conditions (Chahil and Ginsberg 2006, Miao *et al.,* 2021). Abnormal lipid level is one of the contributory factors that hypertension in diabetes patients (Koh and Goh, 2020) and a major risk factor for coronary heart disease and cerebrovascular diseases (Spannella *et al.,* 2019). The Aberration in lipid metabolism in patients suffering from the diabetes-hypertension condition may be attributed to the increase in the amount of free fatty acid released from insulin-resistant fat cells (Chehade *et al.,* 2013; Irondi *et al.,* 2016). Hence, this study shows the significance of evaluating the effects of MOT supplements on the lipid profile of diabetes-hypertension co-morbidity patients.

The change in the serum lipid parameters of (elevated total cholesterol, LDL, triglycerides; and reduced HDL) of the negative control group may be attributed to insulin deficiency and/or resistance in diabetes patients caused as a result of the inactivation of lipoprotein lipase leading to hypertriglyceridemia (Irondi *et al.,* 2016). The observed increase in HDL, reduced triglyceride, total cholesterol and LDL in the group that consumed MOT supplement with the standard drug compared to the other treated group may be attributed to the synergistic effect of the two xenobiotics and hypolipidemic effect of the major constituent of the MOT supplement (Mbikay, 2012; Bao *et al.,* 2020). MOT supplement contains bioactive compounds such as phenolics that important roles in lipid metabolism through the inhibition of lipase and cholesterol esterase (Siasos *et al.,* 2013). Phenolics compound regulate plasma cholesterol concentrations by inhibiting pancreatic cholesterol esterase thus, reducing cholesterol absorption and increasing its faecal excretion (Adisakwattana and Chanathong, 2011).

Increased level of LDL improves the deposition of cholesterol in the blood vessels and therefore increases the chances of the development of cardiovascular diseases in diabetic patients, this is because it facilitates the movement of cholesterol from the liver to body tissues (Pedersen, 2001). The increased HDL, reduced the risk of atherosclerosis, as it enhances the removal and excretion of endogenous cholesterol and cholesteryl esters from the body tissues (Xu *et al*., 2021). The improved lipid profile of the groups fed with MOT supplement is an indication of the potential health benefit of MOT supplement in the management of hyperlipidemia characterising diabetes-hypertension co-morbidity patients.

Examining the activities of liver enzymes and the serum level of albumin, total protein, direct bilirubin and total bilirubin are important parameters in assessing liver function and any clinical/experimental alteration to the liver function (Kalegari *et al.,* 2014). The Elevated blood levels of these enzymes may be related to certain

disease conditions or the effect of toxicants (Singh *et al*., 2001). These marker enzymes are naturally present in high concentration in the cytoplasm, in the cause of liver damage/ injury, these enzymes leak out into the bloodstream (Azab, *et al.,* 2014).

The observed increase in the activities of AST, ALT, and ALP in the negative control patients relative to the positive control patients agrees with the findings of Irondi *et al*., (2016) who reported increased serum activities of AST, ALT, and ALP in diabetic rats. The increase in liver function activities according to Mansour *et al.* (2002) may be due to liver damage caused as a result of oxidative stress effect on liver-specific enzymes causing leakage of these enzymes from the liver into the bloodstream. The observed significant ($p<0.01$) decrease in the activities of the liver enzymes in the MOT-supplemented group relative to the negative control group indicates the hepato-protective effect of the MOT supplement. The significantly lower liver function enzyme activities observed in the MOT-supplemented group in comparison with the standard drug group shows that the supplement may have a high hepato-protective effect than the standard drug. The hepato-protective ability of the supplement may be attributed to its availability of important bioactive constituents with prominent antioxidant activities such as phenolics, β-carotene and vitamin C (Skrovankova *et al*., 2015). These bioactive compounds act as antioxidants by scavenging oxidants such as reactive oxygen species through enzymatic and non-enzymatic reactions (Elemosho *et al.,* 2021). Hence, MOT supplement might be able to ameliorate hepatotoxic consequences caused by diabetes/hypertension, thereby, restoring serum hepatic enzymes.

The reduced plasma protein levels of the diabetic/ hypertensive patients, in comparison with the positive control group, may be ascribed to abnormalities in protein metabolism caused by insulin resistance/deficiency and a decrease in the production of alkaline phosphatase (Gray and Cooper, 2011). Insulin regulates protein metabolism by stimulating protein synthesis and retarding protein degradation (Murray *et al*., 2000). The administration of standard drug and MOT supplements to the patients concurrently and singly significantly, restored the protein level to normal in the diabetic/hypertensive patients.

Albumin is known as an important protein synthesized in the liver and served as an indicator for ascertaining liver function. The result of this study revealed a significant decrease in the albumin level of diabetic/ hypertensive patients compared to the normal group; this indicates liver dysfunction (Debebe *et al.,* 2017). This is because low serum albumin concentration could be attributed to infection or low synthesis of albumin in the liver (Yakubu *et al.,* 2003). Consumption of MOT supplements significantly improves the albumin level in diabetic/hypertensive patients as revealed in this study compared to the negative + standard drug group. This study also suggests that MOT supplement did not cause haemolysis of red blood cells and did not affect uptake and conjugation of bilirubin because the group showed no significant difference in the direct and total bilirubin concentrations.

The kidneys are the major organ involved in the excretion of waste products and the maintenance of homeostasis and acid-base balance in human. Any problem or defect with the kidneys will result in inefficiency in performing their normal function (Imo *et al.,* 2019). Evaluating the serum concentrations of uric acid, creatinine and electrolytes, are important kidney function biochemical indices required to assess the normal function and integrity of the kidney (Abolaji *et al*., 2007). The elevated level of these kidney biomarkers gives insight into the effect of toxicants or xenobiotics on a different parts of the nephrons (Bello *et al.,* 2016).

Measuring serum creatinine, uric and urea is a simple and commonly used indicator for assessing kidney function. The decrease in the creatinine, uric and urea concentration of the given MOT supplement compared to the control groups and the standard drug group shows that it positively affects urea clearance. The use of the standard drug only for the patients shown in this study shows that the drug may have a deleterious effect on the kidney. The alteration in the clearance of creatinine, uric and urea may be attributed to the effects of the supplement consumed and the drug used, thus, affecting the kidney clearance of creatinine, uric and urea. Urea is known as the final degradation product of protein and amino acid metabolism. The elevation of this urea may be attributed to an alteration in the urinary system (Imo *et al.,* 2019). The elevation in creatinine levels in the negative and standard group show that more creatinine was retained in the blood of diabetes/hypertension patients and the patients receiving standard drugs. The significant elevation of creatinine and urea in these patients is an indication of nephrotoxicity (Uhegbu *et al*., 2015).

Blood Electrolytes (sodium, potassium, chloride and bicarbonate) are another good indicator of kidney function. Kidney is an important organ in regulating the level of blood sodium (Na^+) and the amount of Na^+ in the extracellular fluid (Alicic *et al*., 2019). As revealed in this study, there is an elevation in concentrations of serum electrolytes of the group administered with standard

drugs. The consumption of the supplement reduced the concentration of electrolytes in diabetes/hypertension. The concomitant administration of the standard drug and the supplement significantly reduced the serum electrolyte level.

Increase in potassium levels in human can be linked to renal failure, dehydration shock or adrenal insufficiency (Enemor and Okaka, 2013). Chloride is also an important electrolyte for regulating cation/anion in the intra and extracellular fluids (Imo, 2019). Elevated serum chloride concentration in patients that used standard drugs may be attributed to dehydration and defects in the urinary system (Imo, 2019). The alteration in bicarbonate level in the blood may be the result of acid-base imbalance, which may be related to kidney tubular acidosis, and renal failure (Uhegbu *et al*., 2015).

CONCLUSION

The results from this study revealed that supplementing the diet of diabetic and hypertensive co-morbidities patients with *Moringa oleifera* leaf tea might improve the health status of the patients and decrease the side effect of other co-administered drugs.

DECLARAITIONS

Ethics approval and consent to participate

The study design and ethical clearance were approved and granted by the Ethical Committee of Kwara State Ministry of Health, Ilorin, Nigeria, in consonance with the institutional guidelines. An approval number MOH/KS/EU/777/490 was assigned. All the participants gave their informed consent before the commencement of the study.

Consent for publication

Not applicable

Availability of Data and Materials

All data generated and analyzed during this study are included in this published article

Competing interests

The authors declare no conflict of interest.

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Authors' Contributions

Dr. Muinat O. Lambe designed and supervised the laboratory work. Dr. Aliyu. I. Adedo did some of the laboratory works and was involved with the first draft of the manuscript preparation. Mr. Abdulazeez O. Elemosho also did some part of the laboratory work and generated the data, while Dr. Quadri O. Nurudeen supervised, prepared and edited the final draft of the manuscript.

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