Original Research Article

Effects of the Anti-diabetic Polyherbal (Ruzu Bitters) on Glucose, Hepatic and Renal Parameters in Alloxan-induced Diabetic Rats

ABSTRACT

Aim: This study evaluated the effects of the anti-diabetic polyherbal (Ruzu Bitters) on glucose, hepatic and renal parameters in alloxan-induced diabetic rats.

Methodology: A total of 35 male Albino rats weighing between 120-140 g were used for this study. Diabetes was induced by a single intraperitoneal injection of freshly prepared alloxan-monohydrate (140 mg/kg body weight). Fasting plasma glucose (FPG) was determined using the glucose oxidase method. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using Reitman-Frankel method, while alkaline phosphatase (ALP) was determined using the colorimetric phenolphthalein method. The electrolytes, sodium (Na^+), potassium (K^+) and chloride (CL^-) were determined using ion selective electrode (ISE) method. Urea was determined using Urease bertholet method. Creatinine was determined using the Jaffe-Slot method. Phytochemical analysis was done on the herbal mixture, using classical methods.

Results: The results revealed the presence of saponins, alkaloids, flavonoids, polyphenols and tannins in the polyherbal mixture ruzu bitters. FPG levels in the negative control and the treatment groups were significantly lower when compared to the diabetic control. FPG levels were significantly higher in Group 3 and group 4, but showed no significant difference in group 5, compared to the negative control. ALT, AST and ALP levels were significantly higher in the diabetic control and treatment groups, compared to the negative control, except for Group 3 which showed no significant difference. Treatment groups 4 and 5 had significantly higher ALT levels compared to the diabetic control. Also, AST levels in groups 4 and 5 were not significantly different from the diabetic control. Group 4 had significantly higher Na^+ and Cl^- levels compared to both the negative control and diabetic control groups. Urea levels in the diabetic control and all treatment groups were significantly higher than the negative control. Group 3 had significantly lower urea levels, groups 4 and 5 had significantly higher urea levels compared to the diabetic control. Treatment groups 4 and 5 had significantly higher urea and creatinine values compared to the diabetic control.

Conclusion: Administration of 140 mg/kg body weight of alloxan-monohydrate produced significant diabetes in the Albino rats with electrolyte imbalance and elevated urea, creatinine and liver enzyme levels. Treatment with the polyherbal ruzu bitters and glibenclamide had equipotent anti-hyperglycaemic effects. Glibenclamide had hepatoprotective effects on the liver, however, ruzu bitters negatively impacted the liver of the diabetic rats. Also, the combination therapy worsened liver parameters as ruzu bitters reduced the beneficial effects of glibenclamide. Ruzu bitters was nephrotoxic as it exacerbated the renal parameters of the diabetic rats. Authorities should ensure proper evaluation of anti-diabetic herbal products and care should be taken in their use/combination with orthodox drugs, as they could pose public health risk.

Keywords: Diabetes mellitus; hyperglycaemia; complementary and alternative medicine; ruzu bitters; herbal bitters; glibenclamide; liver enzymes; renal function; electrolytes; phytonutrients; alloxan.

1. INTRODUCTION

"Diabetes is a major public health problem, and brings with it, a huge disease burden on the patients. The increased burden of diabetes and the unresolved complications makes most patients resort to complementary and alternative medicine (CAM), in which they practice polypharmacy, combining orthodox and herbal drugs, in an attempt to improve the outcomes of their illnesses as

well as their general well-being" [1, 2]. "The increased prevalence in the use of CAM by diabetic patients in Africa, particularly Nigeria, must be matched with efforts to ascertain the safety as well as the therapeutic efficacies of these drugs. In Africa, especially Nigeria, there are a number of constraints in the control of CAM usage. For instance, there is lack of integration of CAM therapies into African mainstream health care systems. This is despite the World Health Organization (WHO) recommendation to integrate traditional and CAM therapies into national health care systems" [3]. Another major concern is the lack of regulation on CAM use, therefore exposing the population to potential harm. This study looks at the efficacy and safety in the use of an anti-diabetic polyherbal thus evaluated the effects of the anti-diabetic polyherbal (Ruzu Bitters) on glucose, hepatic and renal parameters in alloxan-induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

A total of thirty-five (35) male Albino rats weighing between 120 to 140g were used for the study. The rats were housed in standard cages at regulated room temperature, with controlled 12-hour light-dark cycles, and allowed access to feed and water *ad libitum*. The rats were allowed to acclimatize for two (2) weeks prior to the commencement of study.

2.2 Drugs

A commonly used polyherbal mixture Ruzu bitters was used for the study. Ruzu bitters is manufactured by Ruzu Herbal Products & Services Ltd, Nigeria. Glibenclamide, a standard anti-diabetic drug, was also used for the study. It was manufactured by Glanil Pharmaceuticals, Nigeria.

2.3 Acute Toxicity study

"Acute Toxicity Study was done by the fixed dose procedure [4], using a group of 3 rats. 10 ml/kg body weight of diarth was orally administered to each of the rats. The rats were then observed for signs of toxicity for 48 hours. After observation, there were no signs of toxicity, hence the polyherbal mixture ruzu bitters was deemed safe up to a dose of 10 ml/kg body weight" [4].

2.4 Dose Calculation

2.4.1 Ruzu Bitters

The daily dose was extrapolated from the manufacturers' dose of 120ml/70kg body weight.

A rat of 1kg would require; 1/70 × 120 [4, 5]

= 1.71ml/kg/day.

2.4.2 Glibenclamide

The administered rat dose was extrapolated from the human daily dose [6] as shown below:

Human daily dose is 1 tablet (5mg) twice daily, which is 10mg/day.

Rat dose (mg/kg) = Human daily dose × 0.018 × 5

= 10 × 0.018 × 5

= 0.9mg/kg/day

2.5 Experimental Design and Diabetes Induction

"The rats were weighed and grouped into 5 groups of 7 rats each. Diabetes was induced by a single intraperitoneal injection of freshly prepared alloxan monohydrate (140mg/kg body wt.) dissolved in physiological saline, after a 6 hour fast. Diabetes was confirmed after 48 hours in all the rats, with fasting blood glucose levels above 14mmol/L (250 mg/dl)" [7]. "Treatments (drugs) were administered daily according to the groupings by means of oral gavage for 28 days".[28]

Group 1: Negative control group. Injected only physiological saline intraperitoneally

Group 2: Diabetic control

Group 3: Diabetic rats administered glibenclamide.

Group 4: Diabetic rats administered ruzu bitters.

Group 5: Diabetic rats administered a combination of glibenclamide and ruzu bitters

2.6 Reagents and Biochemical Analyses

"All reagents were commercially purchased and the manufacturer's standard operating procedures were strictly followed. Quality control (QC) samples were run together with the biochemical analysis. Alloxan was gotten from Qualikems Fine Chem Pvt Ltd, India. Fasting plasma glucose (FPG) was determined using Glucose oxidase method" [8], as modified by Randox Laboratories Limited (UK). "The liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using the Reitman-Frankel method" [9], as modified by Randox laboratories limited (UK). "Alkaline phosphatase (ALP) was determined using the Colorimetric endpoint method" [10] as modified by Randox laboratories limited (UK). "The electrolytes, sodium (Na^+), potassium (K^+) and chloride (CL^-) were determined using ion selective electrode (ISE) method" [11]. "Urea was determined using Urease bertholet method" [12], as modified by Randox laboratories limited (UK). "Creatinine was determined using the Jaffe-Slot method" [13], as modified by Randox laboratories limited (UK). "Qualitative phytochemical analysis was done on the herbal mixture using classical methods" [14].

2.7 Statistical Analysis

"Data was analysed using Graph Pad Prism version 8.0.2. Differences among groups were compared using one way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Results were considered statistically significant at 95% confidence interval ($p \leq 0.05$). Values are expressed as Mean ± SD". [28]

3. RESULTS AND DISCUSSION

Table 1: Qualitative Phytochemical Analysis of the Herbal Mixture Ruzu bitters

Phytochemicals	Presence
Saponins	++
Alkaloids	+
Terpenes	-
Coumarins	-
Cardiac glycosides	-
Flavonoids	++
Polyphenols	+
Tannins	++

Phlobatannins	-

+ Present, - Not present

Table 1 shows the results of phytochemical analysis. The results revealed the presence of the phytochemicals saponins, alkaloids, flavonoids, polyphenols and tannins in the polyherbal mixture ruzu bitters in variable amounts. These phytonutrients have modulatory effects on diabetic pathways and could be responsible for the alteration of biochemical parameters [15].

Table 2: Effects of Treatment on Fasting Plasma Glucose (FPG) and Liver Enzymes of the Rats.

Groups	FPG (mmol/L)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Group 1 (Neg Control)	5.40 ± 0.52^{b}	8.60 ± 0.79^{b}	42.63 ± 5.81^{b}	46.86 ± 2.27^{b}
Group 2 (Pos Control)	17.18 ± 2.71^{a}	12.37 ± 1.55^{a}	61.10 ± 4.57^{a}	71.90 ± 4.49^{a}
Group 3 (Gli)	$7.65 \pm 0.93^{a\ b}$	9.50 ± 1.43^{b}	45.86 ± 6.53^{b}	43.41 ± 3.53^{b}
Group 4 (Ruzu)	$7.52 \pm 0.44^{a\ b}$	$34.17 \pm 1.30^{a\ b}$	57.40 ± 6.93^{a}	$61.02 \pm 5.07^{a\ b}$
Group 5 (Gli + Ruzu)	7.13 ± 0.31^{b}	$29.96 \pm 1.44^{a\ b}$	60.19 ± 6.34^{a}	70.13 ± 3.73^{a}
p-value	< 0.0001	< 0.0001	0.0006	< 0.0001
F-value	68.80	225.9	7.019	47.00
Remark	S	S	S	S

S – Significant, ^{a} – significantly different from negative control, ^{b} – significantly different from positive control, Gli – Glibenclamide, Ruzu – Ruzu Bitters.

Table 2 shows the results of fasting plasma glucose (FPG), the liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) after treatment. FPG levels in the negative control and the treatment groups were significantly lower ($P< .05$) when compared to the diabetic control. FPG levels were significantly higher ($P< .05$) in Group 3 (administered glibenclamide) and group 4 (administered Ruzu bitters), but showed no significant difference ($P> .05$) in group 5 (the combination group), compared to the negative control. This implies administration of glibenclamide and ruzu bitters as singular therapies reduced the FPG levels of the rats from diabetic levels. However, the single therapies were not effective enough to return the FPG values to baseline control levels. It also implies the combination therapy was more effective than the singular therapies, and showed synergistic

action in reducing FPG levels down to baseline control values. Phytonutrients in herbal drugs act alone or in interaction with orthodox drugs, affecting metabolic pathways and bringing about different glycemic responses. In a similar study, ethanolic extract of *Carica papaya* leaves showed comparable anti-hyperglycaemic activity when administered alongside glibenclamide in alloxan-induced diabetic rats [16]. The results also agree with the works of Briggs *et al*. [17], in which the combination therapy of glibenclamide and the polyherbal drug diarth significantly reduced glucose levels, and was more effective compared to glibenclamide administered alone, in alloxan-induced diabetic rats.

ALT levels were significantly higher ($P< .05$) in the diabetic control and treatment groups, compared to the negative control, except for Group 3 (glibenclamide) which showed no significant difference ($P> .05$). This implies the induction of diabetes significantly elevated ALT levels in the rats. However, treatment with glibenclamide was effective and reduced ALT levels to normal control levels. Treatment groups 4 (ruzu bitters) and 5 (combination group) had significantly higher ($P< .05$) ALT levels compared to the diabetic control. This implies treatment with the herbal bitters singularly and in combination with glibenclamide worsened ALT values, significantly elevating it even above the diabetic control levels.

AST levels were significantly higher ($P< .05$) in the diabetic control and treatment groups 4 (ruzu bitters) and 5 (combination group), except for group 3 (glibenclamide) that showed no significant difference ($P> .05$) compared to the negative control. This implies diabetes elevated AST levels, and treatment with glibenclamide was effective in reducing AST levels to normal baseline levels. There were no significant differences between groups 4 (ruzu bitters) and 5 (combination group) when compared to the diabetic control. This implies treatment with ruzu bitters, and its combination with glibenclamide was not effective and had no impact on AST levels.

ALP levels were significantly higher ($P< .05$) in the diabetic control and treatment groups 4 and 5, except for group 3 that showed no significant difference ($P> .05$) compared to the negative control. This indicates the elevated ALP levels were reduced to normal control levels by treatment with glibenclamide. There was no significant difference in ALP levels in group 5 when compared to the diabetic control. This also indicates treatment with ruzu bitters was not as effective as glibenclamide, and the combination therapy had no impact on ALP levels. The

results from the liver enzymes imply glibenclamide improved the liver condition in the diabetic rats and had hepatoprotective effects. Administration of ruzu bitters negatively impacted the liver, and its combination with glibenclamide demonstrated antagonistic drug-herb effects, in which the effect of the combination was less effective than that from the singular administration of glibenclamide. The liver has been associated with diabetes related oxidative stress. Injury to the liver is a common occurrence in patients with uncontrolled diabetes, with biochemical, histopathological and physiological changes [18]. The results are in consonance with the works of Khajuria *et al*. [19], in which experimental induced diabetes significantly elevated levels of transaminases (AST and ALT) and phosphatases (ALP and ACP) in rats. The results also agree with the works of Briggs *et al*. [20], in which administration of glibenclamide had hepatoprotective effects in the diabetic rats. However, in their work the combination of glibenclamide and the herbal capsule glucoblock, was synergistic and reduced levels of the liver enzymes to baseline control values. In another work by Gidado *et al*. [21], administration of glibenclamide offered hepatoprotective effects and lowered liver enzyme levels. In the same work however, *Senna occidentalis* leaf supplement increased the activities of all the liver enzymes, caused hepatocellular necrosis, sinusoidal congestion and hemorrhage as revealed in the histopathological studies of the liver.

Table 3: Effects of Treatment on Urea, Creatinine and Electrolyte Values of the Rats after Treatment

Groups	Na^+ (mmol/L)	Cl^- (mmol/L)	K^+ (mmol/L)	Urea (mmol/L)	Creatinine (µmol/L)
Group 1 (Neg Control)	150.7 ± 2.9	101.7 ± 1.7[b]	4.4 ± 0.2	3.56 ± 0.25[b]	75.77 ± 3.88[b]
Group 2 (Pos Control)	151.0 ± 2.5	105.5 ± 0.9[a]	4.8 ± 0.1	7.52 ± 0.44[a]	86. 80 ± 2.63[a]
Group 3 (Gli)	153.0 ± 1.9	102.8 ± 1.6	4.5 ± 0.3	6.19 ± 0.72[a b]	78.61 ± 2.48[b]
Group 4 (Ruzu)	156.4 ± 1.2[a b]	115.5 ± 1.5[a b]	4.6 ± 0.2	11.75 ± 0.58[a b]	107.1 ± 4.90[a b]
Group 5 (Gli + Ruzu)	151.0 ± 1.2	104.5 ± 1.5	5.1 ± 0.2[a]	13.12 ± 0.48[a b]	145.3 ± 4.02[a b]
p-value	0.0047	< 0.0001	0.0003	< 0.0001	< 0.0001
F-value	5.078	74.82	8.460	152.4	289.5
Remark	S	S	S	S	S

S – Significant, [a] – significantly different from negative control, [b] – significantly different versus positive control, Gli – Glibenclamide, Ruzu – Ruzu Bitters.

Table 3 shows the results of Electrolytes, Urea and Creatinine of the rats after treatment. There were no significant differences ($P > .05$) in sodium levels, except for group 4 (administered ruzu bitters) which was significantly higher ($P < .05$) than both the diabetic and negative controls. Chloride levels were significantly higher ($P < .05$) in the diabetic control against the negative control. There were no significant differences ($P > .05$) in chloride in the treatment groups, except group 4 (administered ruzu bitters) which was significantly higher than both the negative and diabetic controls. There were no significant differences ($P > .05$) in potassium levels except in group 5 (the combination group) which was significantly higher ($P < .05$) than the negative control. This implies ruzu bitters had nephrotoxic properties, impacting sodium and chloride levels, and the combination with glibenclamide elevating potassium levels above the baseline control levels.

Urea levels in the diabetic control and all treatment groups were significantly higher ($P < .05$) than the negative control. Indicating diabetes increased the urea levels, and no treatment was effective in returning urea levels to baseline control values. Group 3 (administered glibenclamide) had significantly lower ($P < .05$) urea levels, however groups 4 (administered ruzu bitters) and 5 (the combination group) were significantly higher compared to the diabetic control. This implies diabetes not only negatively impacted the kidneys, but the administration of ruzu bitters and its combination with glibenclamide were toxic to the kidneys, grossly elevating urea levels even above the diabetic levels. Creatinine levels in the diabetic control and all treat groups were significantly higher ($P < .05$), except for group 3 (administered glibenclamide) which had no significant difference ($P > .05$) when compared to the negative control. Treatment groups 4 (administered ruzu bitters) and 5 (the combination group) had significantly higher creatinine values compared to the diabetic control. This is similar to the result of the urea levels, indicating administration of ruzu bitters and the combination with glibenclamide was noxious to the kidneys, increasing creatinine levels.

Electrolyte abnormalities and imbalances are frequent occurrences in diabetes, with impaired renal function and acid-base disorders also contributory factors [22, 23]. The results are in

consonance with the works of Engwa *et al*. [24], in which they found significantly elevated potassium in diabetics. Sodium, chloride, urea and creatinine levels were also high though not significant. In another study by Preetha *et al*. [25], treatment with coconut water and glibenclamide improved urea and creatinine levels in alloxan induced diabetic rats. Patrick-Iwuanyanwu & Nkpaa, [26], found electrolyte imbalances (hypernatremia and hypokalaemia) and altered haematological indices in albino rats administered Class Bitters®-a polyherbal mixture. In their study, they concluded that long-term administration of Class Bitters® might cause renal disease and anemia. In another study, Navya *et al*. [27] reported that the extract of *Syzygium cumini* and *Momordica charantia* showed better efficiency in restoring the electrolyte imbalance as compared to the orthodox drug metformin.

4. CONCLUSION

Administration of 140 mg/kg body weight of alloxan-monohydrate produced significant diabetes in the albino rats with elevated liver enzyme levels. There was electrolyte imbalance in the diabetic rats with significant elevations in urea and creatinine levels. Treatment with the polyherbal mixture ruzu bitters and glibenclamide had equipotent effects in reducing fasting plasma glucose levels. Treatment with glibenclamide was hepatoprotective as it reduced the elevated ALT, AST and ALP levels in the diabetic rats. Treatment with the polyherbal ruzu bitters negatively impacted the liver of the diabetic rats. The combination therapy worsened liver parameters as ruzu bitters reduced the beneficial effects of glibenclamide. Ruzu bitters was nephrotoxic as it exacerbated the renal parameters of the diabetic rats. Herbal mixtures are not harmless and safe as perceived. Authorities should ensure proper evaluation of anti- diabetic herbal products and care should be taken in their use/combination with orthodox drugs. Their efficacy should be assessed before they are allowed into the markets as some may not meet their claims and pose public health risk acting alone or through drug-herb interactions.

Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1) International Diabetes Federation. International Diabetes Federation Diabetes Atlas (7th Ed.). International Diabetes Federation; 2016.
2) Medagama AB, Bandara R. The use of Complementary and Alternative Medicines (CAMs) in the treatment of diabetes mellitus: Is continued use safe and effective? *Nutrition Journal. 2014;*13:102.
3) Matheka DM, Demaio AR. Complementary and alternative medicine use among diabetic patients in Africa: A Kenyan perspective. *Pan African Medical Journal*. 2013;15(110):1-5.
4) Organisation for Economic Co-operation and Development. Guidance Document on Acute Oral Toxicity Testing: Environmental health and safety monograph series on testing and assessment No.24. 2001;24. [Accessed 14th July, 2018] Available: https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd-gd24.pdf
5) Erhirhie EO, Ekene NE, Ajaghaku DL. Guidelines on dosage calculation and stock solution preparation in experimental animals' studies. *Journal of Natural Sciences Research*. 2014;4(18):100–105.
6) Paget GE, Barnes JM. Evaluation of drug activities. In Lawrence, D. R & Bacharach, A. L. (Eds.). Pharmacometrics (pp. 161). New York: Academy Press; 1964.
7) Deeds MC, Anderson JM, Armstrong AS, Gastineau DA, Hiddinga HJ, Jahangir A, Eberhardt NL, Kudva YC. Single dose streptozotocin induced diabetes: Considerations for study design in islet transplantation models. *Lab Animal*. 2011; 45(3):131–140.
8) Barham D, Trinder P. An improved colourreagent for the determination of blood glucose by the oxidase system. Analyst. 1972;97(151):142-145.
9) Reitman S, Frankel S. A colorimetric method for the determination of glutamic oxaloacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*. 1957;28:56-66.
10) Klein B, Read PA, Babson LA. Alkaline phosphatase activity measurement. *Clinical chemistry*. 1960;6:269-275.
11) Buck R, Lindner E. Recommendations for nomenclature of ionselective electrodes (IUPAC Recommendations 1994). *Pure and Applied Chemistry*. 1994;66(12):2527-2536. https://doi.org/10.1351/pac199466122527
12) Bretaudiere JP, Phung HT, Bailly M. Direct enzymatic determination of urea in plasma and urine with a centrifugal analyzer. *Clinical Chemistry*. 1976;22(10):1614-1617
13) Slot C. Plasma creatinine determination. A new and specific Jaffe reaction method. Scandinavian *Journal of Clinical and Laboratory Investigation.* 1965;17(4):381-387. doi:10.3109/00365516509077065
14) Ezeonu CS, Ejikeme CM. Qualitative and quantitative determination of phytochemical contents of indigenous Nigerian softwoods. *New Journal of Science*. 2016;2016: 5601327.

15) Wink M. Modes of action of herbal medicines and plant secondary metabolites. *Medicines*. 2015;2(3):251-286.
16) Airaodion AI, Ogbuagu EO, Ekenjoku JA Ogbuagu U. Antidiabetic effect of ethanolic extract of carica papaya leaves in alloxan-induced diabetic rats., *American Journal of Biomedical Science and Research*. 2019;5(3). MS.ID.000917. doi:10.34297/AJBSR.2019.05.000917
17) Briggs ON, Elechi-amadi KN, Okobia C, Ezeiruaku FC. Anti-hyperglycaemic, anti-dyslipidaemic and hepatoprotective effects of the polyherbal mixture diarth in alloxaninduced diabetic rats. *Journal of Complementary and Alternative Medical Research*. 2020;9(3):51-57.
18) Farokhi F, Farkhad NK, Togmechi A, Soltani BK. Preventive effects of Prangos ferulacea (L.) Lindle on liver damage of diabetic rats induced by alloxan. *Avicenna Journal of Phytomedicine*. 2011;2:63-71.
19) Khajuria P, Raghuwanshi P, Rastogi A, Koul AL, Zargar R, Kour S. Hepatoprotective effect of Seabuckthorn-leaf extract in streptozotocin induced -diabetes mellitus in Wistar rats. Indian Journal of Animal Research. 2018;52(12): 1745-1750.
20) Briggs ON, Elechi-Amadi KN, Ezeiruaku FC, Teme RE. Lipidaemic and hepatic status of type 2 diabetic rats treated with the polyherbal capsule glucoblock. *Journal of Advances in Medical and Pharmaceutical Sciences*. 2020;22(1):6-15. DOI:https://doi.org/10.9734/jamps/2020/v22i130150
21) Gidado NM, Tanko Y, Sada NH, Mohammed A. Effects of *Senna occidentalis* leaf supplement on blood glucose level, liver enzymes and total protein in alloxan-induced diabetic wistar rats. *Bayero Journal of Pure and Applied* Sciences. 2016;9(1): 68 – 75. http://dx.doi.org/10.4314/bajopas.v9i1.11
22) Elisaf MS, Tsatsoulis AA, Katopodis KP, Siamopoulos KC. Acid-base and electrolyte disturbances in patients with diabetic ketoacidosis. *Diabetes Research and Clinical Practice.* 1996;34:23–27.
23) Liamis G, Liberopoulos E, Barkas F, Elisaf M. Diabetes mellitus and electrolyte disorders. *World Journal of Clinical Cases*. 2014;2(10): 488–496. doi: 10.12998/wjcc.v2.i10.488
24) Engwa GA, Nwalo FN, Attama TA, Abonyi MC, Akaniro-Ejim EN, Unachukwu MN, Njokunwogbu AN, Ubi BE. Influence of type 2 diabetes on serum electrolytes and renal function indices in patients. *Journal of Clinical and Diagnostic Research*. 2018;12(6):13-16. doi: 10.7860/JCDR/2018/35940.11673.
25) Preetha PP, Girija DV, Rajamohan T. Comparative effects of mature coconut water (*Cocos nucifera*) and glibenclamide on some biochemical parameters in alloxan induced diabetic rats. *Revista Brasileira de Farmacognosia*, 2013;23(3):481-487. https://doi.org/10.1590/S0102-695X2013005000027
26) Patrick-Iwuanyanwu KC, Nkpaa KW. Toxicity effect of sub-chronic oral administration of class bitters® - a polyherbal formula on serum electrolytes and haematological indices in male wistar albino rats. *Journal of Xenobiotics*. 2015;5(1):5369. doi:10.4081/xeno.2015.5369
27) Navya G, Shirisha Y, Girija P, Venkateshwarlu K, Sirisha K. Effect of *Momordica charantia* and Syzygium cumini extract on serum electrolytes in alloxan induced diabetic rats. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2018;10(11):24-27. 10.22159/ijpps.2018v10i11.24963.

28) Briggs ON, Elechi-Amadi KN, Okobia C, Ezeiruaku FC. Anti-hyperglycaemic, Anti-dyslipidaemic and Hepatoprotective Effects of the Polyherbal Mixture Diarth in Alloxan-induced Diabetic Rats. Journal of Complementary and Alternative Medical Research. 2020;9(3):51-7.