Effect of Ruzu Herbal Bitters on the kidney Function and Hematological Parameters of Alloxan-Induced Diabetic Rats

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Abstract: The aim of the study was to investigate the effect of Ruzu herbal bitters (RHB) on some kidney function and hematological parameters of alloxan-induced diabetic rats. Fifty-four adult albino rats were divided into nine groups of six rats each. Group 1 was the normal control. Groups 2–6 were diabetic. Group 2 was not treated while groups 3 - 6 were respectively treated with 0.5 mg/kg b.w of glibenclamide, 0.14, 0.29 and 0.57 ml/kg b.w of RHB. Groups 7 - 9 were not diabetic but treated as groups 4 - 6. The treatment lasted for 21 days. The results showed significant ($p<0.05$) increase in the blood glucose level and significant ($p<0.05$) decrease in weight in diabetic-untreated group compared to the normal control. The kidney function and hematological parameters tested showed significant ($p<0.05$) increases in the levels of urea, creatinine, calcium, chloride and platelets; with significant decreases ($p<0.05$) in the levels of sodium, potassium, magnesium, bicarbonate, packed cell volume (PCV), hemoglobin (Hb), white blood cells (WBC) and red blood cells (RBC) in diabetic-untreated group compared to the normal group. However, treatment of the diabetic rats with different doses of RHB caused the reversal of these effects to near normal levels in a dose dependent manner. The highest effect was observed in the group treated with 0.57 ml/kg b.w of RHB, which was equipotent with 0.5 mg/kg b.w of glibenclamide, a standard antidiabetic drug. Therefore, our study suggests that RHB has antidiabetic and nephroprotective effects, hence, recommended for the management of diabetes mellitus.

Key words: blood glucose level, body weight, diabetes mellitus, hematological parameters, kidney function, Ruzu herbal bitters (RHB), spleen histology.

1. INTRODUCTION

Diabetes mellitus (DM) is a pathological and metabolic disorder caused by abnormal insulin action, especially insulin insensitivity, with glucose intolerance and hyperglycemia being common features to all types [1]. DM is the most common endocrine disorder that affects more than 100 million people worldwide (6 % population). It is caused by deficiency or ineffective production of insulin in the pancreas resulting in high or low level of glucose in the blood [2]. Chronic hyperglycemia caused by DM is associated with long-term damage, dysfunction or failure of some organs, such as the eyes, kidneys, nerves, heart, and blood vessels [3]. There are two major types of diabetes mellitus: Type 1 DM also known as insulin dependent diabetes mellitus (IDDM) and Type 2 DM also known as non-insulin dependent diabetes mellitus (NIDDM). Type 1 diabetes mellitus is caused by insulin deficiency due to destruction of pancreatic β-cells by an autoimmune reaction that can be triggered by different factors [4]. Destruction of pancreatic β-cells progresses to absolute deficiency in insulin. This condition develops rapidly in young people and has been found to occur in any age group [4]. Type 2 diabetes mellitus is characterized by insulin resistance in the peripheral tissue and an insulin secretory defect of β-cells. This is the most common form of diabetes mellitus and is highly associated with a family history of diabetes, old age, obesity and lack of exercise [5]. It is more common in women, especially women with a history of gestational diabetes. Type 2 diabetes is characterized by derangement of carbohydrate, protein and fat metabolism [6]. Insulin resistance and hyperinsulinemia eventually leads to impaired glucose tolerance [7].

Diabetes mellitus is associated with an increased risk of cardiovascular disease mediated via oxidative stress. Reactive oxygen species can directly damage lipids, proteins or DNA and modulate intracellular signaling pathways [8]. Symptoms of DM include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision [3]. Diabetes mellitus causes increased risk of many complications such as cardiovascular diseases (CVD), peripheral vascular disease (PVD), coronary artery disease (CAD), stroke, neuropathy, nephropathy (renal failure), retinopathy, amputations, and blindness [9], [10].

Biguanides and sulfonylureas are some of the hypoglycemic agents used for treatment of diabetes. The main disadvantage of currently available synthetic drugs is that they have to be taken for life-time and also, they produce numerous side effects [11]. Medicinal plants and their bioactive constituents can be used for treatment of DM throughout the world especially in places where

access to the conventional anti-DM drugs are not available or inadequate [12]. Therefore, apart from being the primary producers in the food chain, plants are also the primary sources of therapeutic agents [13]. Furthermore, there is a traditional believe that a combination of plants or their extracts will give a better therapeutic efficacy for a particular disease or multiple diseases than that of a single plant because the individual plant contains different therapeutic agents in which when combined together will give a better result than that of a single plant [13]. Therefore, most herbal preparations work in synergy, leading to the emergence of polyherbal mixtures, such as Ruzu herbal bitters.

Ruzu herbal bitters (RHB) is a poly-herbal mixture consisting of *Curculigo pilosa* root (40 %), *Uvaria chamae* stem (20 %) and *Citrullus colocynthis* bark (40%). It is produced in Nigeria by Ruzu Natural Health Product and Services, with a NAFDAC Registration Number: A7-1102L. The mixture is in liquid form in a well packaged bottled and commercially available in Nigeria. Ruzu herbal bitters (RHB) is indicated to have the following functions amongst others: antidiabetic, antihyperlipidemic, antioxidant, anti-inflammatory, analgesic, antibacterial, antifungal, laxative and hair growth promoting properties. Due to the medicinal claims of the manufacturers without much scientific evidences, there is the need to carry out scientific investigations to ascertain the efficacy of RHB in the management of diabetes mellitus and its associated kidney problem, including its effect on hematological parameters of diabetic rats.

2. MATERIALS AND METHODS

2.1 Materials and Equipment: Ruzu herbal bitters (RHB) was purchased from the producer and used directly, spectrophotometer (Spectro 21D PEC MEDICALS USA), incubator, weighing balance, measuring cylinder, glass wares (pyrex), centrifuge (Binatone), refrigerator, sample containers. All the chemicals and reagents used in this research were of the purest analytical grade commercially available.

Figure 1. Ruzu herbal bitters. Dosage: Adults – 2-4 tablespoons 1 or 2 times daily.
Children – 1 tablespoon (5 ml) once in 3 days or as directed by physician (Producer).

2.2 Methods

Animal Management: Adult male albino rats (weighing between 96 and 121 g) were purchased from the animal house of the Department of Zoology, University of Nigeria, Nsukka and acclimatized for one week prior to commencement of the experiment. They were kept at room temperature and maintained *ad libitum* on water and feed; weighed prior to commencement of experiment and weekly till the end of the experiment.

Induction of Diabetes: Rats were fasted overnight and experimental diabetes was induced by intraperitoneal injection of freshly prepared alloxan with a single dose of 100 mg/kg body weight. After three days, rats with blood glucose level greater than 200 mg/dl (hyperglycemia) were selected for the experiment [4]. The one-touch blood glucose monitoring meter (glucometer) and test strips were used for the assay.

Experimental Design: Dosages of 0.14, 0.29 and 0.57 ml/kg body weight of RHB, equivalent to 10, 20 and 40 ml of RHB/70 kg body weight adult daily, as prescribed by the producer, were chosen for the study. Fifty-four (54) albino rats were divided into nine groups of six rats each as follows:

(a) Group 1: Normal control rats (non-diabetic).
(b) Group 2: Positive control rats (diabetic untreated).
(c) Group 3: Standard control rats (diabetic) treated with 0.5 mg/kg body weight of glibenclamide [14].
(d) Group 4: Diabetic rats treated with 0.14 ml/kg body weight of RHB.
(e) Group 5: Diabetic rats treated with 0.29 ml/kg body weight of RHB.
(f) Group 6: Diabetic rats treated with 0.57 ml/kg body weight of RHB.
(g) Group7: Non-diabetic rats treated with 0.14 ml/kg body weight of RHB
(h) Group 8: Non-diabetic rats treated with 0.29 ml/kg body weight of RHB
(i) Group 9: Non-diabetic rats treated with 0.57 ml/kg body weight of RHB.

The baseline blood glucose levels of the rats were determined before the induction of diabetes. The treatment, weight and blood glucose determinations (7 days' intervals) lasted for 21 days; after which the rats were sacrificed and blood samples collected via ocular and cardiac puncture on the first day of post-treatment for biochemical analyses. Some of the blood samples were allowed to clot in anticoagulant free bottles and centrifuged at 3000 rpm for 15 minutes for kidney function assays; while some of the blood samples were collected in EDTA-anticoagulant bottles for hematological assays. Also, sections of the kidney and spleen from the rats in the experimental groups were collected for histological examination.

Determination of Blood Glucose Level

The blood glucose levels of the rats were determined using a glucometer (blood glucose meter). The Accu-Check strip was inserted into the indicated area of the glucometer and allowed to show light at the point where a drop of blood would be placed. A lancet was used to puncture the tail end of the rat. The tail was pressed to bring out blood which was then placed on the indicated portion of the test strip in the glucometer. The blood glucose level which showed on the screen of the glucometer (in mg/dl) was recorded.

Determination of Kidney Function Parameters

1. Estimation of urea concentration using Randox kits by the method of Tietz [15].
2. Estimation of creatinine concentration using Randox kits by the Method of Bartels and Bohmer [16].
3. Estimation of sodium concentration using Micropoint Diagnostic kits according to the method of Tietz [17].
4. Estimation potassium concentration using Micropoint Diagnostic kits according to the method of Tietz [17].
5. Estimation of calcium concentration using Randox kits by the method of Ray Srkar and Chauhan [18].
6. Estimation of magnesium concentration using Prestige Diagnostics kits by the method of Tietz [19].
7. Estimation chloride concentration using Micropoint Diagnostic kits according to the method of Tietz *et al.* [20].
8. Estimation of carbon dioxide concentration using Teco Diagnostics kits by the method of Forrester *et al.* [21].

Determination of Hematological Parameters by the method of Ochei and Kolhatkar [22]

1. Estimation of packed cell volume (PCV).
2. Determination of haemoglobin (Hb) concentration.
3. Determination of erythrocyte (red blood cell, RBC) count by haemocytometry.
4. Determination of total leucocyte (white blood cell, WBC) count by haemocytometry.
5. Determination of total platelet (PLAT) count by haemocytometry.

Histological Examinations of the kidney and Spleen

Sections of the kidney and spleen collected from the rats in the experimental groups were fixed in 10 % phosphate buffered formalin for a minimum of 48 hours. The tissues were subsequently trimmed, dehydrated in 4 grades of alcohol (70%, 80%, 90%

and absolute alcohol), cleared in 3 grades of xylene and embedded in molten wax. On solidifying, the blocks were sectioned, 5µm thick with a rotary microtome, floated in water bathe and incubated at 60 ˚C for 30 minutes. The 5µm thick sectioned tissues were subsequently cleared in 3 grades of xylene and rehydrated in 3 grades of alcohol (90 %, 80 % and 70 %). The sections were then stained with hematoxylin for 15 minutes. Blueing was done with ammonium chloride. Differentiation was done with 1 % acid alcohol before counterstaining with Eosin. Permanent mounts were made on degreased glass slides using a mountant; DPX. The prepared slides were examined with a Motic™ compound light microscope using x4, x10 and x40 objective lenses. The photomicrographs were taken using a Motic™ 5.0 megapixels microscope camera at x160 and x400 magnifications.

2.3 Statistical Analysis

The data obtained from the laboratory tests was subjected to one-way analysis of variance (ANOVA). Significant differences were obtained at $P \leq 0.05$ and the results were expressed as mean ± standard error of mean (SEM). The SPSS version 20 and Microsoft excel 2007 software were used.

3. RESULTS

3.1 Blood Glucose Level

Table 1 shows the effect of RHB on the blood glucose levels (mg/dl) of the rats. Administration of alloxan caused a significant ($p < 0.05$) increase in the blood glucose levels (BGL) of the rats when compared to their baseline BGL. There was a significant ($p < 0.05$) increase in the BGL of the diabetic-untreated group from day 0 of the baseline (70.00 ± 66.97) to diabetes induction (241.25 ± 36.42), and progressively to the day 21 (514.3 ± 8.06); unlike the normal control group where there was a gradual increase in the BGL from day 0 (78.50 ± 4.80) to the day 21 (88.25 ± 2.06) of the experiment. However, treatment of the diabetic groups 4, 5 and 6 with 0.14, 0.29 and 0.57 ml/kg b.w of RHB respectively, resulted in significant ($p < 0.05$) decreases in their BGL in a dose dependent manner. Also, for the non-diabetic groups 7, 8 and 9 treated with 0.14, 0.29 and 0.57 ml/kg b.w of RHB respectively; there was no significant ($p < 0.05$) decrease in the blood glucose levels in group 7 (79.25 ± 9.11 to 75.50 ± 4.93) but there were significant ($p < 0.05$) decreases in groups 8 (84.33 ± 8.02 to 70.33 ± 4.16) and 9 (81. 33 ± 5.03 to 66.33 ± 1.15) within the 21 days of treatment. Therefore, the result showed that RHB has hypoglycemic/antidiabetic property.

Table 1. Effect of Ruzu herbal bitters (RHB) on the blood glucose concentrations of alloxan-induced diabetic rats

	Blood glucose levels in mg/dl expressed in mean ± S.D				
GROUPS	DAY 0	DIABETES (DAY 0)	DAY 7	DAY 14	DAY 21
GROUP 1	78.50 ± 4.80^{a}		86.25 ± 5.19^{ab}	83.69 ± 9.00^{ab}	88.25 ± 2.06^{b}
GROUP 2	70.00 ± 66.97^{a}	241.25 ± 36.42^{b}	294.75 ± 7.14^{c}	379.70 ± 3.39^{d}	514.3 ± 8.06^{e}
GROUP 3	76.00 ± 11.79^{a}	384.00 ± 6.00^{c}	159.67 ± 10.60^{b}	91.33 ± 17.92^{a}	72.33 ± 14.57^{a}
GROUP 4	70.33 ± 2.08^{a}	296.67 ± 147.55^{b}	134.00 ± 34.51^{a}	96.00 ± 6.00^{a}	79.67 ± 15.04^{a}
GROUP 5	84.00 ± 6.08^{a}	304.67 ± 21.08^{c}	157.66 ± 8.96^{b}	103.33 ± 8.08^{a}	81.00 ± 14.52^{a}
GROUP 6	68.00 ± 5.20^{a}	330.67 ± 167.16^{c}	183.67 ± 6.42^{b}	103. 33 ± 10.97^{a}	62.67 ± 9.71^{a}
GROUP 7	79.25 ± 9.11^{a}		84.75 ± 5.68^{a}	81.75 ± 4.79^{a}	75.50 ± 4.93^{a}
GROUP 8	84.33 ± 8.02^{b}		82.00 ± 3.46^{ab}	76.00 ± 7.94^{ab}	70.33 ± 4.16^{a}
GROUP 9	81. 33 ± 5.03^{c}		77.33 ± 4.73^{bc}	71.33 ± 6.85^{ab}	66.33 ± 1.15^{a}

Mean values with different letters as superscripts across the rows are considered significant ($p < 0.05$)

Group 1: normal control; Group 2: diabetic - untreated; Group 3: diabetic treated with 0.5 mg/kg b.w. of glibenclamide;
Group 4: diabetic treated with 0.14 ml/kg b.w. of RHB; Group 5: diabetic treated with 0.29 ml/kg b.w. of RHB;
Group 6: diabetic treated with 0.57 ml/kg b.w. of RHB; Group 7: non-diabetic treated with 0.14 ml/kg b.w. of RHB;
Group 8: non-diabetic treated with 0.29 ml/kg b.w. of RHB; Group 9: non-diabetic treated with 0.57 ml/kg b.w. of RHB.
DAY 0 = baseline blood glucose level (before induction of diabetes); DIABETES (DAY 0) = blood glucose level after induction of diabetes

3.2 Body Weight

Table 2 shows the effect of Ruzu herbal bitters (RHB) on the body weight (g) of alloxan-induced diabetic rats. There was a significant ($p < 0.05$) increase in the body weight of the normal control group from day 0 (117.03 ± 15.14) to the day 21 (139.27 ± 14.38) of the experiment. The body weight of the diabetic-untreated group showed significant ($p < 0.05$) decrease from day 0 before induction of diabetes (104.57 ± 28.25), after induction of diabetes (99.00 ± 23.19 to the day 21 (75.10 ± 5.66) of the experiment. For the diabetic groups 4, 5 and 6 treated with 0.14, 0.29 and 0.57 ml/kg b.w of RHB respectively, there were significant ($p < 0.05$) increases in the body weight from day 0 to day 21 in a dose dependent manner; which is comparable to that observed in the diabetic group 3 treated with 0.5 mg/kg b.w. of glibenclamide. Also, for the non-diabetic groups 7, 8 and 9 treated with 0.14, 0.29 and 0.57 ml/kg b.w of RHB respectively, there was significant ($p < 0.05$) increases in the body weight from day 0 to the day 21 of treatment comparable to that of the normal control. Our result showed that RHB could help in weight gain in diabetic rats.

Table 2. Effects of Ruzu herbal bitters (RHB) on the body weights of alloxan-induced diabetic rats

	Body weights in g expressed in mean ± S.D				
GROUPS	DAY 0	DIABETES (DAY 0)	DAY 7	DAY 14	DAY 21
GROUP 1	117.03 ± 15.14^{a}		122.10 ± 12.83^{a}	128 35 ± 15. 10^{b}	139.27 ± 14.38^{c}
GROUP 2	104.57 ± 28.25^{d}	99.00 ± 23.19^{cd}	94.97 ± 18.93^{c}	83.67 ± 12. 34^{b}	75.10 ± 5.66^{a}
GROUP 3	119.37 ± 9.26^{a}	121.80 ± 9.31^{a}	125.86 ± 4.30^{a}	140.53 ± 11.09^{b}	151.60 ± 10.53^{c}
GROUP 4	101.20 ± 0.31^{a}	108.20 ± 9.49^{a}	114.67 ± 13.55^{ab}	121.73 ± 5.27^{bc}	132.73 ± 21.07^{c}
GROUP 5	113.87 ± 7.24^{a}	116.40 ± 8.13^{a}	122.67 ± 6.81^{ab}	129.33 ± 1.53^{bc}	134.00 ± 1.48^{c}
GROUP 6	121.07 ± 9.62^{a}	131.57 ± 3.38^{b}	135.17 ± 2.58^{bc}	141.17 ± 2.05^{c}	144.97 ± 2.97^{d}
GROUP 7	111.55 ± 14.16^{a}		117.90 ± 14.26^{a}	127.05 ± 9.30^{ab}	137.15 ± 11 .43^{b}
GROUP 8	100.64 ± 15.13^{a}		112.88 ± 11.12^{a}	128.93 ± 13.04^{b}	131.10 ± 12.58^{b}
GROUP 9	96.44 ± 18.25^{a}		116.85 ± 9.29^{b}	129.50 ± 13.56^{b}	136.23 ± 9.80^{b}

Mean values with different letters as superscripts across the rows are considered significant ($p < 0.05$)

Group 1: normal control; Group 2: diabetic - untreated; Group 3: diabetic treated with 0.5 mg/kg b.w. of glibenclamide;
Group 4: diabetic treated with 0.14 ml/kg b.w. of RHB; Group 5: diabetic treated with 0.29 ml/kg b.w. of RHB;
Group 6: diabetic treated with 0.57 ml/kg b.w. of RHB; Group 7: non-diabetic treated with 0.14 ml/kg b.w. of RHB;
Group 8: non-diabetic treated with 0.29 ml/kg b.w. of RHB; Group 9: non-diabetic treated with 0.57 ml/kg b.w. of RHB.

DAY 0 = baseline blood glucose level (before induction of diabetes); DIABETES (DAY 0) = blood glucose level after induction of diabetes

3.3 Kidney Function Parameters

Table 3 shows the effect of Ruzu herbal bitters (RHB) on some serum kidney function markers of alloxan-induced diabetic rats.

Urea Concentration (mmol/dl)

There was a significant ($p < 0.05$) increase in urea concentration (mmol/dl) observed in the diabetic untreated group (47.40 ± 1.25) compared to that of the normal control group (37.27 ± 0.91). Significant decrease ($p < 0.05$) was observed in the RHB treated diabetic groups 4, 5 and 6 in a dose dependent manner. The urea concentration of group 6 treated with 0.57 ml/kg b.w of RHB (38.04 ± 0.37) was comparable with that of diabetic group 3 treated with 0.5 mg/kg b.w. of glibenclamide (38.14 ± 0.47); which was not significantly different with that of the normal control group. There was a gradual decrease with no significant ($p < 0.05$) difference in the urea concentrations of the different doses of RHB treated non-diabetic groups 7 (37.14 ± 0.59), 8 (37.06 ± 0.45) and 9 (36.95 ±0.59) in a dose dependent manner compared to that of the normal control group.

Creatinine (CREAT) Concentration (mg/dl)

As shown in Table 3, creatinine concentration (mg/dl) increased significantly ($p < 0.05$) in the diabetic untreated group (1.87 ± 0.45) compared to that of the normal control group (1.17 ± 0.06). The creatinine concentration decreased significantly ($p < 0.05$) in the RHB treated diabetic groups 4, 5 and 6 in a dose dependent manner. However, the result of group 6 treated with 0.57 ml/kg b.w of RHB (1.29 ± 0.06) showed no significant ($p < 0.05$) difference with that of diabetic group 3 treated with 0.5 mg/kg b.w. of glibenclamide (1.30 ± 0.06). There was no significant ($p < 0.05$) difference in the creatinine concentrations of the RHB treated non-diabetic groups 7 (1.16 ± 0.03), 8 (1.14 ± 0.03) and 9 (1.13 ± 0.07), which are comparable with that of the normal control group.

Sodium (Na^+) Concentration (mEq/L)

There was a significant ($p < 0.05$) decrease in the concentration of sodium observed in the diabetic untreated group (134.37 ±3.42) compared to that of the normal control group (165.63 ± 3.42). Significant ($p < 0.05$) increase in the concentration of sodium was observed in the RHB treated diabetic groups in a dose dependent manner. The result of group 6 treated with 0.57 ml/kg b.w of RHB (153.13 ± 3.42) was comparable with that of diabetic group 3 treated with 0.5 mg/kg b.w. of glibenclamide (156.25 ± 6.85). There was a gradual increase in the concentrations of sodium of the RHB treated non-diabetic groups 7 (162.50 ± 6.85), 8 (168.75 ± 6.85) and 9 (171.86 ± 3.42); which are significantly ($p < 0.05$) different from that of the normal control group.

Potassium (K^+) Concentration (mEq/L)

There was a significant ($p < 0.05$) decrease in the potassium concentration in the diabetic untreated group (1.55 ± 0.09) compared to that of the normal control group (4.60 ± 0.27). Howver, significant increase ($p < 0.05$) in potassium concentration was observed in the RHB treated diabetic groups 4, 5 and 6 in a dose dependent manner. No significant ($p < 0.05$) difference was observed in the results of groups 6 and 3 treated respectively with 0.57 ml/kg b.w of RHB (4.00 ± 0.03) and 0.5 mg/kg b.w. of glibenclamide (3.94 ± 0.03). However, there was a gradual increase in the potassium concentrations of the RHB treated non-diabetic groups 7 (4.51 ± 0.36), 8 (4.97 ± 0.27) and 9 (5.06 ± 0.60) in a dose dependent manner when compared with that of the normal control group.

Calcium (Ca^{2+}) Concentration (mg/dl)

Calcium concentration (mg/dl) increased significantly ($p < 0.05$) in the diabetic untreated group (20.36 ± 1.59) compared to that of the normal control group (13.82 ± 0.79). The calcium concentration decreased significantly ($p < 0.05$) in the RHB treated diabetic groups 4, 5 and 6 in a dose dependent manner. There was no significant ($p < 0.05$) difference in the calcium concentrations of group 6 (14.18 ± 0.39) and group 3 (14.55 ± 1.59). Likewise, there was no significant ($p < 0.05$) difference in the calcium concentrations of the RHB treated non-diabetic groups 7 (13.46 ± 0.40), 8 (12.73 ± 1.20) and 9 (12.72 ± 2.79). Hence, groups 3, 6, 7, 8 and 9 were not significantly ($p < 0.05$) different from that of the normal control group.

Magnesium (Mg^{2+}) Concentration (mg/dl)

The concentration of magnesium [Mg^{2+}] was significantly ($p < 0.05$) decreased in the diabetic untreated group (1.60 ± 0.08) compared to that of the normal control group (3.13 ± 0.38). Significant increase ($p < 0.05$) in [Mg^{2+}] was observed in the RHB treated diabetic groups 4, 5 and 6 in a dose dependent manner. No significant ($p < 0.05$) difference was observed in the results of group 6 (2.92 ± 0.30) and group 3 (2.78 ± 0.31). While there was a gradual increase in [Mg^{2+}] in the RHB treated non-diabetic groups 7 (3.13 ± 0.07), 8 (3.27 ± 0.38) and 9 (3.34 ± 0.30); which are comparable with that of the normal control group.

Chloride ion (Cl^-) Concentration (mEq/L)

Chloride ion concentration [Cl^-] increased significantly ($p < 0.05$) in the diabetic untreated group (98.05 ± 0.71) compared to that of the normal control group (91.75 ± 0.61). Treatment of the diabetic groups with different doses of RHB caused significantly ($p < 0.05$) decrease in the [Cl^-] in a dose dependent manner. The result of group 6 treated with 0.57 ml/kg b.w of RHB (92.86 ± 0.71) is comparable with that of diabetic group 3 treated with 0.5 mg/kg b.w. of glibenclamide (93.13 ± 1.14). There was no significant difference ($p < 0.05$) in the [Cl^-] of the RHB treated non-diabetic groups 7 (92.21 ± 0.48), 8 (92.64 ± 0.95) and 9 (92.86 ± 0.71).

Bicarbonate ion (HCO_3^-) Concentration (mmol/dl)

There was a significant ($p < 0.05$) decrease in the bicarbonate (HCO_3^-) concentration in the diabetic untreated group (28.35 ± 0.18) compared to that of the normal control group (30.72 ± 0.13). Significant increase ($p < 0.05$) in HCO_3^- concentration was observed in the RHB treated diabetic groups 4, 5 and 6 in a dose dependent manner. Also, the result of group 6 treated with 0.57 ml/kg b.w of RHB (30.37 ± 0.09) is comparable with that of group 3 treated with 0.5 mg/kg b.w. of glibenclamide (30.35 ± 0.15). There was a gradual increase in HCO_3^- concentrations in the RHB treated non-diabetic groups 7 (30.48 ± 0.14), 8 (30.58 ± 0.33) and 9 (30.71 ± 0.08).

Table 3. Effect of Ruzu herbal bitters (RHB) on some serum kidney function markers of alloxan-induced diabetic rats

	Kidney function markers expressed in mean ± S.D							
GROUPS	UREA (mmol/dl)	CREAT (mg/dl)	Na^+ (mEq/L)	K^+ (mEq/L)	Ca^{2+} (mg/dl)	Mg^{2+}(mg/dl)	Cl^- (mEq/L)	HCO_3^- (mmol/dl)
GROUP 1	37.27 ± 0.91^{ab}	1.17 ± 0.06^{a}	165.63 ± 3.42^{ef}	4.60 ± 0.27^{e}	13.82 ± 0.79^{a}	3.13 ± 0.38^{ef}	91.75 ± 0.61^{a}	30.72 ± 0.13^{f}
GROUP 2	47.40 ± 1.25^{d}	1.87 ± 0.45^{d}	134.37 ± 3.42^{a}	1.55 ± 0.09^{a}	20.36 ± 1.59^{c}	1.60 ± 0.08^{a}	98.05 ± 0.71^{e}	28.35 ± 0.18^{a}
GROUP 3	38.14 ± 0.47^{b}	1.30 ± 0.06^{ab}	156.25 ± 6.85^{d}	3.94 ± 0.03^{d}	14.55 ± 1.59^{a}	2.78 ± 0.31^{d}	93.13 ± 1.14^{b}	30.35 ± 0.15^{d}
GROUP 4	40.69 ± 0.56^{c}	1.62 ± 0.12^{c}	140.63 ± 3.42^{b}	2.12 ± 0.30^{b}	18.54 ± 1.19^{b}	1.95 ± 0.15^{b}	96.32 ± 0.71^{d}	28.96 ± 0.16^{b}
GROUP 5	39.90 ± 0.81^{c}	1.46 ± 0.04^{bc}	148.88 ± 1.23^{c}	3.43 ± 0.12^{c}	16.73 ± 2.39^{b}	2.29 ± 0.08^{c}	95.24 ± 0.47^{c}	29.46 ± 0.23^{c}
GROUP 6	38.04 ± 0.37^{b}	1.29 ± 0.06^{ab}	153.13 ± 3.42^{cd}	4.00 ± 0.03^{d}	14.18 ± 0.39^{a}	2.92 ± 0.30^{de}	92.86 ± 0.71^{b}	30.37 ± 0.09^{de}
GROUP 7	37.14 ± 0.59^{a}	1.16 ± 0.03^{a}	162.50 ± 6.85^{e}	4.51 ± 0.36^{e}	13.46 ± 0.40^{a}	3.13 ± 0.07^{ef}	92.21 ± 0.48^{ab}	30.48 ± 0.14^{de}
GROUP 8	37.06 ± 0.45^{a}	1.14 ± 0.03^{a}	168.75 ± 6.85^{fg}	4.97 ± 0.27^{f}	12.73 ± 1.20^{a}	3.27 ± 0.38^{f}	92.64 ± 0.95^{ab}	30.58 ± 0.33^{ef}
GROUP 9	36.95 ± 0.59^{a}	1.13 ± 0.07^{a}	171.86 ± 3.42^{g}	5.06 ± 0.60^{f}	12.72 ± 2.79^{a}	3.34 ± 0.30^{f}	92.86 ± 0.71^{b}	30.71 ± 0.08^{f}

Mean values with different letters as superscripts down the columns are considered significant ($p < 0.05$)

Group 1: normal control; Group 2: diabetic - untreated; Group 3: diabetic treated with 0.5 mg/kg b.w. of glibenclamide;
Group 4: diabetic treated with 0.14 ml/kg b.w. of RHB; Group 5: diabetic treated with 0.29 ml/kg b.w. of RHB;
Group 6: diabetic treated with 0.57 ml/kg b.w. of RHB; Group 7: non-diabetic treated with 0.14 ml/kg b.w. of RHB;
Group 8: non-diabetic treated with 0.29 ml/kg b.w. of RHB; Group 9: non-diabetic treated with 0.57 ml/kg b.w. of RHB.
CREAT (creatinine), Na^+ (sodium ion), K^+ (potassium ion), Ca^{2+} (calcium ion), Mg^{2+}(magnesium ion), Cl^- (chloride ion), HCO_3^- (bicarbonate ion)

3.3 Hematological Parameters

Table 4 shows the effect of Ruzu herbal bitters (RHB) on some hematological parameters of alloxan-induced diabetic rats.

Packed Cell Volume (PCV) Level (%)

The PCV level of the diabetic untreated group (44.00 ± 0.67) decreased significantly ($p < 0.05$) compared to that of the normal control group (61.67 ± 1.25). There was a gradual increase but no significant ($p < 0.05$) difference in the PCV levels in the RHB treated diabetic groups 4 (54.50 ± 0.90), 5 (55.15 ± 0.88) and 6 (56.18 ± 1.07) in a dose dependent manner. In contrast, there was a gradual decrease in the PCV levels in the RHB treated non-diabetic groups 7 (56.41 ± 1.17), 8 (55.51 ±1.17) and 9 (55.00 ± 1.06) in a dose dependent manner.

Hemoglobin (Hb) Level (mg/dl)

There was a significant ($p < 0.05$) decrease in the Hb level observed in the diabetic untreated group (20.27 ± 0.29) compared to that of the normal control group (26.51 ± 1.25). No significant ($p < 0.05$) difference but gradual increase was observed in the Hb levels of the RHB treated diabetic groups 4 (24.36 ± 1.01), 5 (24.92 ± 0.46) and 6 (25.12 ± 0.91), including group 3 treated with glibenclamide (25.20 ± 0.70). Likewise, there was no significant ($p < 0.05$) difference but gradual decrease in the Hb levels of the RHB treated non-diabetic groups 7 (25.02 ± 0.58), 8 (24.80 ± 0.58) and 9 (24.78 ± 1.15) in a dose dependent manner.

White Blood Cell (WBC) Count ($x\ 10^3/\mu l$**)**

The WBC count of the diabetic untreated group (3.11 ± 0.07) decreased significantly ($p < 0.05$) compared to that of the normal control group (4.78 ± 0.18). There was a gradual increase with no significant ($p < 0.05$) difference in the WBC count in the RHB treated diabetic groups 4 (4.06 ± 0.31), 5 (4.18 ± 0.40) and 6 (4.35 ± 0.36) in a dose dependent manner; including that of group 3 (4.40 ± 0.31). In contrast, there was a gradual decrease with no significant ($p < 0.05$) difference in the WBC count of the RHB treated non-diabetic groups 7 (4.56 ± 0.48), 8 (4.42 ± 0.36) and 9 (4.20 ± 0.55) in a dose dependent manner.

Red Blood Cell (RBC) Count ($x\ 10^6/\mu l$**)**

The RBC count of the diabetic untreated group (3.70 ± 0.12) decreased but with no significant ($p < 0.05$) difference compared to that of the normal control group (3.95 ± 0.13). There was a gradual increase but no significant ($p < 0.05$) difference in the RBC count of the RHB treated diabetic groups 4 (3.70 ± 0.10), 5 (3.82 ± 0.23) and 6 (3.85 ± 0.24), with the diabetic group 3 treated with glibenclamide (3.90 ± 0.26). Also, there was no significant ($p < 0.05$) difference in the RBC count of the RHB treated non-diabetic groups 7 (3.92 ± 0.34), 8 (3.96 ± 0.17) and 9 (3.94 ± 0.35).

Platelets (PLAT) Count ($x\ 10^4/\mu l$**)**

There was a significant ($p < 0.05$) increase in the platelets count observed in the diabetic untreated group (2.51 ± 0.26) compared to that of the normal control group (2.01 ± 0.16). However, there was a gradual decrease with no significant ($p < 0.05$) difference in the platelets count observed in the RHB treated diabetic groups 4 (2.27 ± 0.32), 5 (2.15 ± 0.21) and 6 (2.06 ± 0.37) in a dose dependent manner, comparable to that of group 3 treated (2.10 ± 0.23). Gradual decrease with no significant ($p < 0.05$) difference in the platelets count was observed in the RHB treated non-diabetic groups 7 (2.01 ± 0.11), 8 (1.98 ± 0.04) and 9 (1.96 ± 0.08).

Table 4. Effects of Ruzu herbal bitters (RHB) on some Hematological Indices of alloxan-induced diabetic rats

	Hematological Parameters expressed in mean ± S.D				
GROUPS	PCV (%)	Hb (g/dl)	WBC (x 10^3/µl)	RBC (x 10^6/µl)	PLAT (x 10^4/µl)
GROUP 1	61.67 ± 1.25^{f}	26.51 ± 1.25^{c}	4.78 ± 0.18^{d}	3.95 ± 0.13^{a}	2.01 ± 0.16^{a}
GROUP 2	44.00 ± 0.67^{a}	20.27 ± 0.29^{a}	3.11 ± 0.07^{a}	3.70 ± 0.12^{a}	2.51 ± 0.26^{c}
GROUP 3	58.50 ± 1.38^{e}	25.20 ± 0.70^{b}	4.40 ± 0.31^{bcd}	3.90 ± 0.26^{a}	2.10 ± 0.23^{ab}
GROUP 4	54.50 ± 0.90^{b}	24.36 ± 1.01^{b}	4.06 ± 0.31^{b}	3.70 ± 0.10^{a}	2.27 ± 0.32^{bc}
GROUP 5	55.15 ± 0.88^{bcd}	24.92 ± 0.46^{b}	4.18 ± 0.40^{bc}	3.82 ± 0.23^{a}	2.15 ± 0.21^{ab}
GROUP 6	56.18 ± I.07^{cd}	25.12 ± 0.91^{b}	4.35 ± 0.36^{bcd}	3.85 ± 0.24^{a}	2.06 ± 0.37^{ab}
GROUP 7	56.41 ± 1.17^{d}	25.02 ± 0.58^{b}	4.56 ± 0.48^{cd}	3.92 ± 0.34^{a}	2.01 ± 0.11^{ab}
GROUP 8	55.51 ±1.17^{bcd}	24.80 ± 0.58^{b}	4.42 ± 0.36^{bcd}	3.96 ± 0.17^{a}	1.98 ± 0.04^{a}
GROUP 9	55.00 ± 1.06^{bc}	24.78 ± 1.15^{b}	4.20 ± 0.55^{bc}	3.94 ± 0.35^{a}	1.96 ± 0.08^{a}

Mean values with different letters as superscripts down the columns are considered significant ($p < 0.05$)

Group 1: normal control; Group 2: diabetic - untreated; Group 3: diabetic treated with 0.5 mg/kg b.w. of glibenclamide;
Group 4: diabetic treated with 0.14 ml/kg b.w. of RHB; Group 5: diabetic treated with 0.29 ml/kg b.w. of RHB;
Group 6: diabetic treated with 0.57 ml/kg b.w. of RHB; Group 7: non-diabetic treated with 0.14 ml/kg b.w. of RHB;
Group 8: non-diabetic treated with 0.29 ml/kg b.w. of RHB; Group 9: non-diabetic treated with 0.57 ml/kg b.w. of RHB.
PCV = packed cell volume, Hb = hemoglobin, WBC = white blood cell, RBC = red blood cell, PLAT = platelet

Histological Examination of Kidney

The effect of Ruzu herbal bitters (RHB) on the histology of the kidneys of the different groups of alloxan-induced diabetic and non-diabetic rats are presented in Figure 2 (a and b). Sections of the kidney collected from the normal control group showed the normal renal histomorphological features of the animals (Plate 1). The section showed normal glomeruli (G), in bowman's capsules (white arrows) surrounded by numerous renal tubules (proximal convoluted tubules, pars recta, distal convoluted tubules and collecting ducts) suspended in a highly vascularised connective tissue meshwork (renal interstitium) and renal tubules (black arrows). Section of the kidney collected from the diabetic untreated group (Plate 2) showed severe degeneration and necrosis of the renal tubules with infiltration of inflammatory mononuclear leukocytes into the renal interstitium (white arrow). Variably thickening of the Bowman's capsules were observed. Some of the Bowman's space showed the presence of eosinophillic casts and some of the relatively normal renal tubules showed accumulation of eosinophillic tubular casts (blue arrows) inside the lumen. Similar to group 2, the group 4 (Plate 4) treated with 0.14 ml/kg b.w. of RHB showed eosinophillic tubular casts in the renal tubules (white arrows). The tubules in the cortex and medulla were affected. Eosinophillic casts were also observed in the Bowman's spaces (blue arrow). However, the diabetic groups 5 and 6 treated with 0.29 and 0.57 ml/kg b.w. of RHB (Plates 5 and 6) respectively, including the diabetic group 3 (Plate 3) treated with 0.5 mg/kg b.w. of glibenclamide, showed normal renal histomorphology, almost the same with that of the normal control group. Also, all the non-diabetic groups treated with 0.14, 0.29 and 0.57 ml/kg b.w. of RHB (Plates 7, 8 and 9) respectively, showed normal renal histomorphology, almost the same with that of the normal control group.

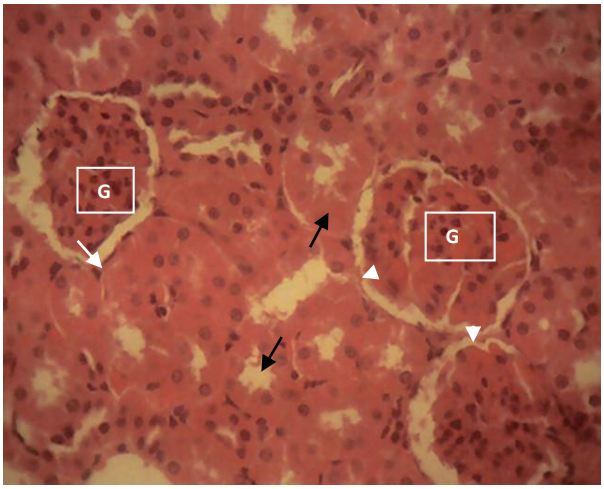

Plate 1. GROUP 1 (H & E x 400)

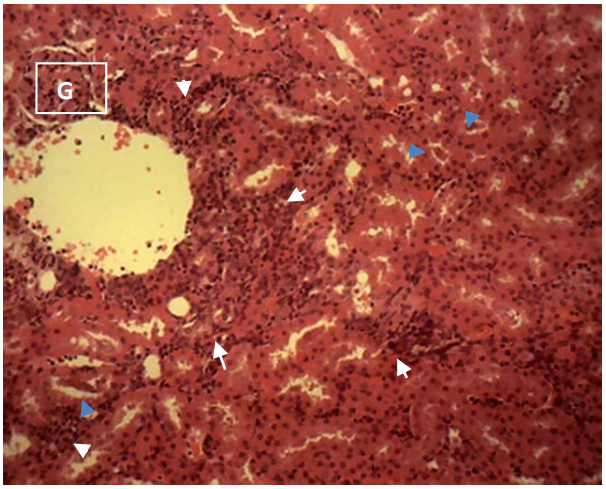

Plate 2. GROUP 2 (H & E x 160)

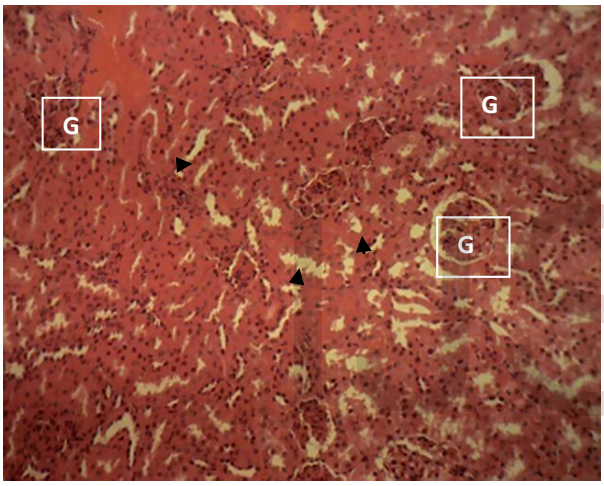

Plate 3. GROUP 3 (H & E x 160)

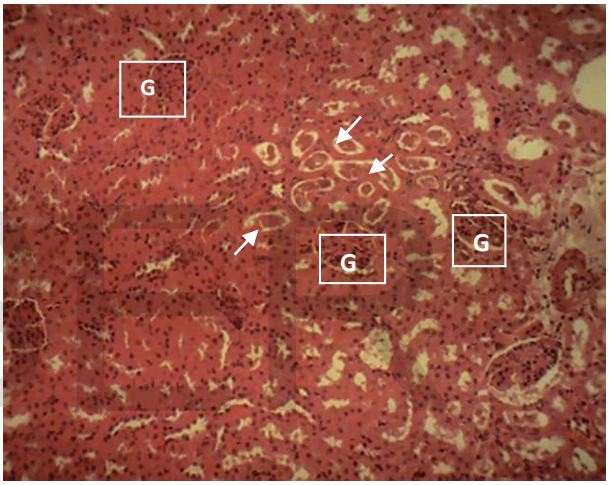

Plate 4. GROUP 4 (H & E x 160)

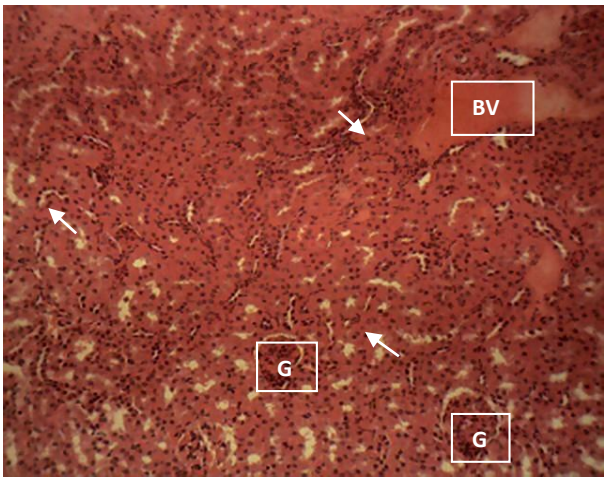

Plate 5. GROUP 5 (H & E x 160)

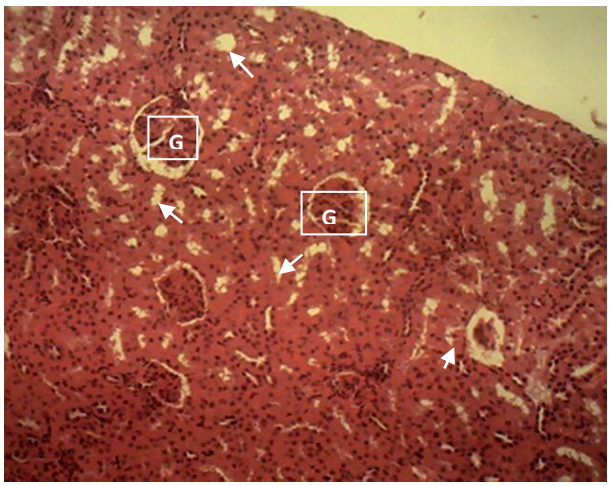

Plate 6. GROUP 6 (H & E x 160)

Figure 2a. Showing the histology of kidney sections: **(Plate 1)** - normal renal histomorphological features - normal Glomeruli (G) in Bowman's capsules (white arrows) surrounded by numerous renal tubules (black arrows); **(Plate 2)** - severe degeneration and necrosis of the renal tubules with infiltration of inflammatory mononuclear leukocytes into the renal interstitium (white arrows), with variably thickening of the Bowman's capsules and renal tubular casts (blue arrows); **(Plate 3)** - normal renal histomorphology as in plate A; **(Plate 4)** - eosinophilic tubular casts in the renal tubules (white arrow) and Bowman's spaces (blue arrows), the tubules in the cortex and medulla were affected; **(Plates 5 and 6)** - normal renal histomorphology as in plate A

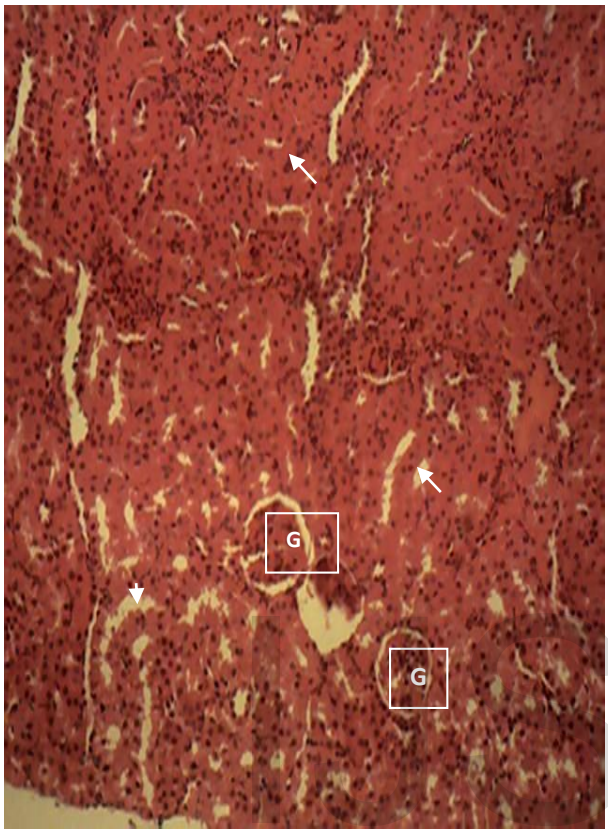

Plate 7. GROUP 7 (H & E x 160)

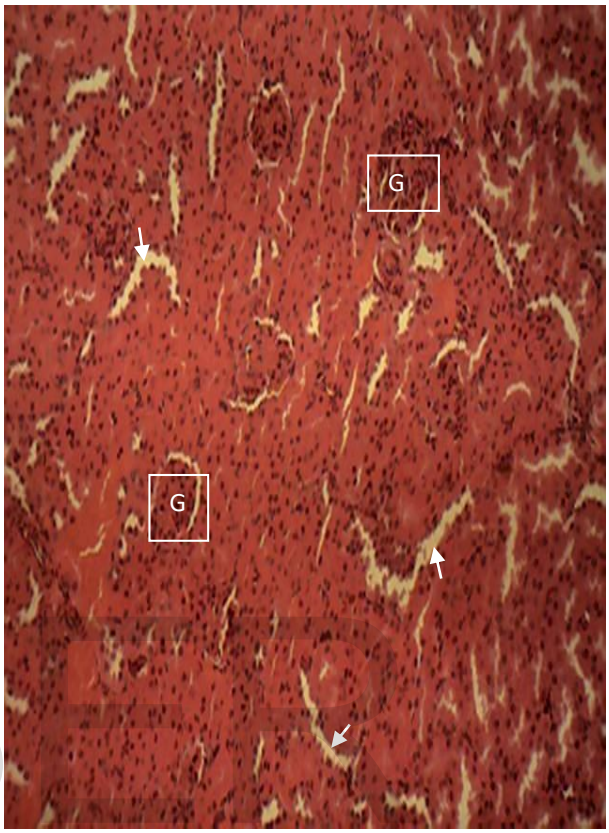

Plate 8. GROUP 8 (H & E x 160)

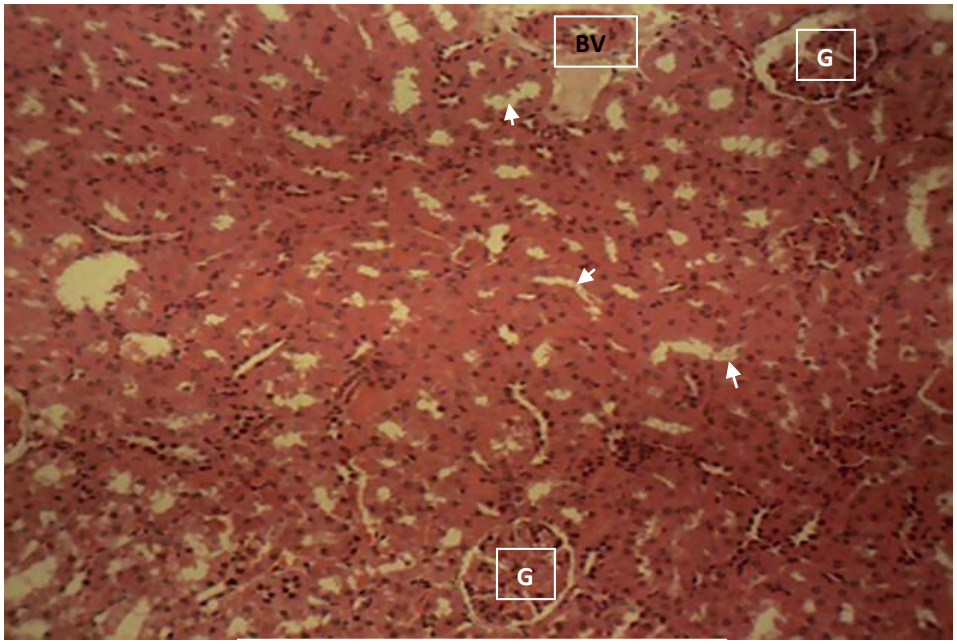

Plate 9. GROUP 9 (H & E x 160)

Figure 2b. Showing the histology of kidney sections of non-diabetic rats treated with 0.14 ml/kg b.w. (**G**), 0.29 ml/kg b.w. (**H**) and 0.57 ml/kg b.w. (**I**) of Ruzu herbal bitters: all the groups showed normal renal histomorphology similar to plate A.

Histological Examination of the Spleen

The effect of Ruzu herbal bitters (RHB) on the histology of the spleen of alloxan-induced diabetic rats presented in Figure 3 (a and b). Sections of the spleen collected from the animals in all the groups (Plates 1 – 9), including the diabetic untreated group, showed normal splenic histomorphology. Each of the section showed normal white (W) and red (R) pulps. The white pulps were made up of predominantly small lymphocytes in the cortical region and large lymphocytes in the medullar areas. The white pulp contains the sinusoids which receive and remove senescent red blood cells (H & E x 160).

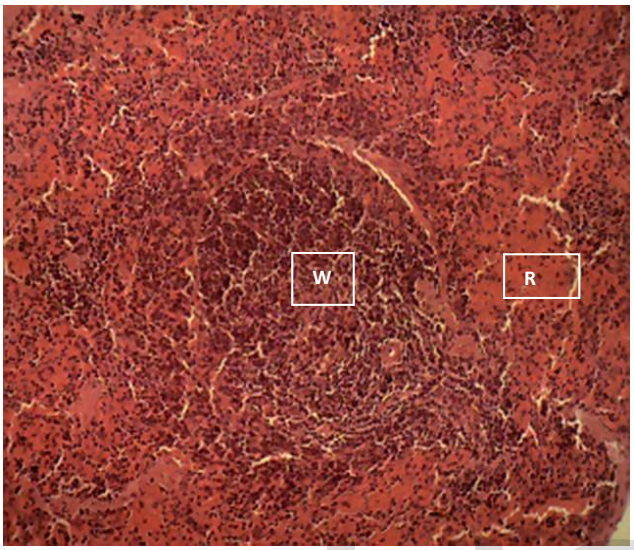

Plate 1. GROUP 1 (H & E x 160)

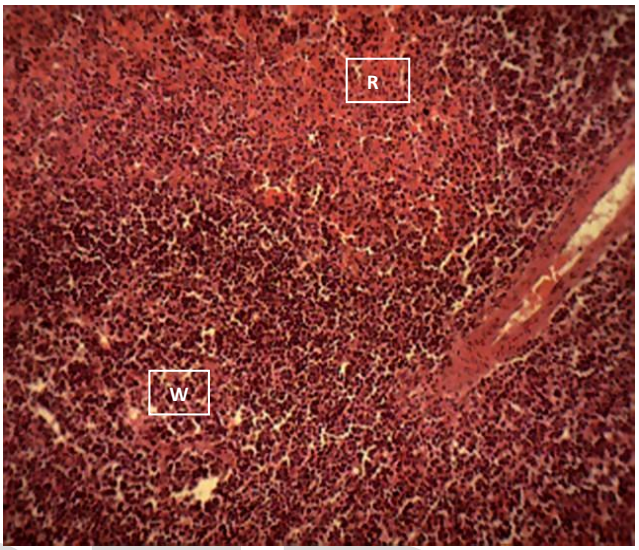

Plate 2. GROUP 2 (H & E x 160)

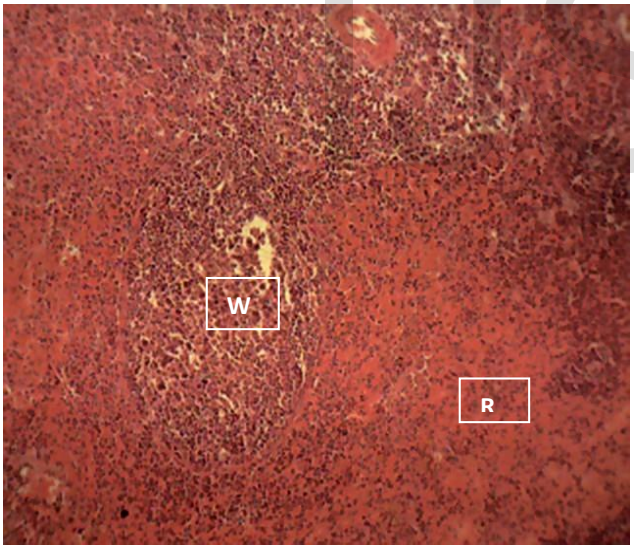

Plate 3. GROUP 3 (H & E x 160)

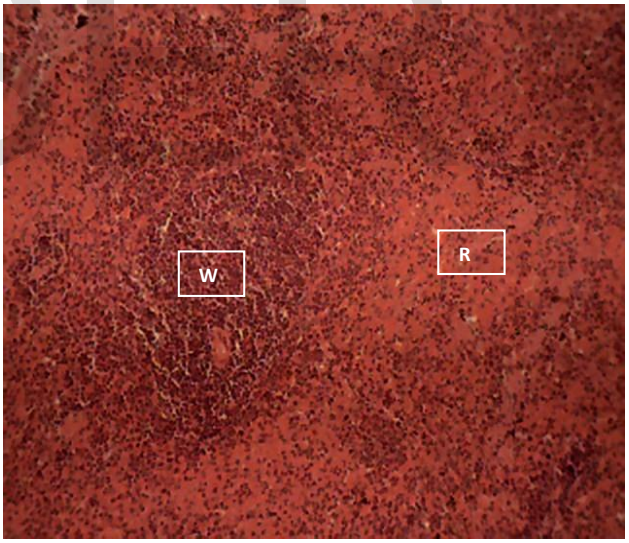

Plate 4. GROUP 4 (H & E x 160)

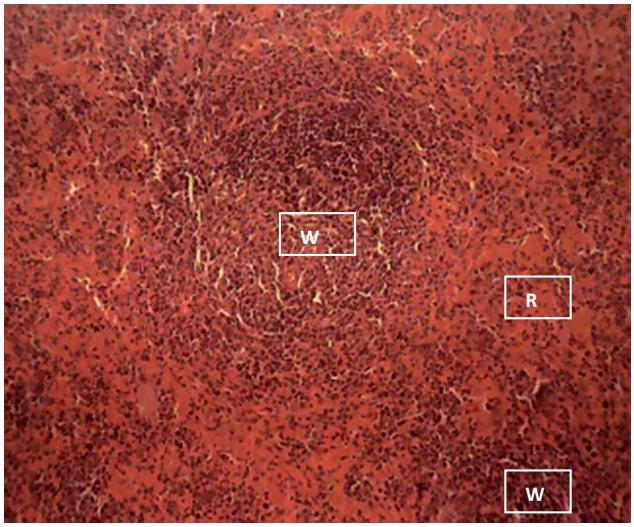

Plate 5. GROUP 5 (H & E x 160)

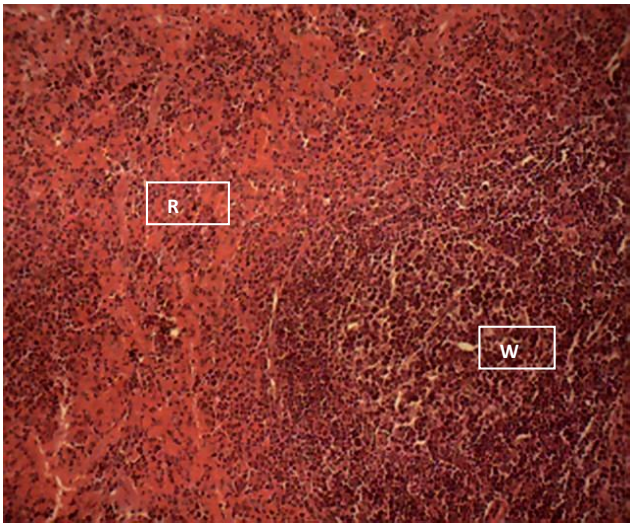

Plate 6. GROUP 6 (H & E x 160)

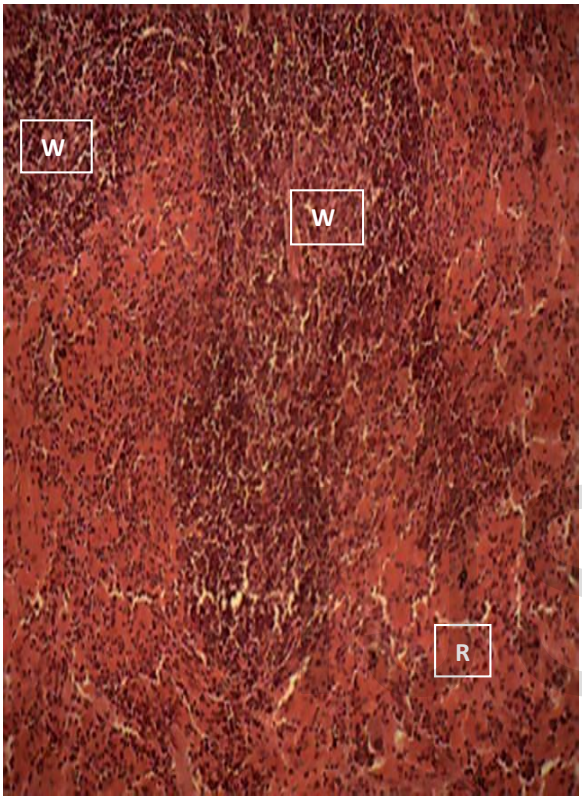

Plate 7. GROUP 7 (H & E x 160)

Plate 8. GROUP 8 (H & E x 160)

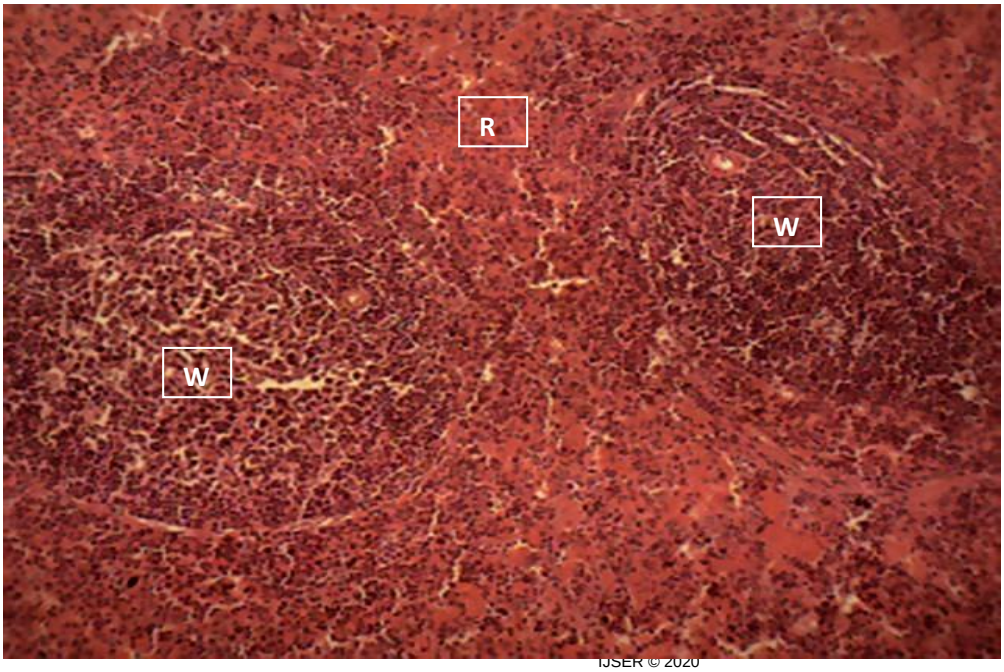

Plate 9. GROUP 9 (H & E x 160)

Figure 3b. Histology of spleen sections of non – diabetic rats treated with 0.14 ml/kg b.w. (**Plate 7**), 0.29 ml/kg b.w. (**Plate 8**) and 0.57 ml/kg b.w. (**Plate 9**) of Ruzu herbal bitters; each of the groups

4. DISCUSSION

Effect of RHB on the Blood Glucose Level

There was a significant ($p < 0.05$) increase in the blood glucose levels of the rats after a single dose of 100 mg/kg body weight alloxan injection. The increase in glucose levels could be as a result of alloxan-induced reactive oxygen species, including increased cytosolic calcium concentrations that led to a rapid destruction of pancreatic islet cells leading to reduction in the synthesis and release of insulin [23], [24].

There was a consistent significant ($p < 0.05$) increase in the blood glucose level of the diabetic-untreated group after diabetes induction to the last day of the experiment, unlike in the normal control group. However, treatment of the diabetic rats with different doses of Ruzu herbal bitters (RHB) resulted in a significant ($p < 0.05$) reduction in their blood glucose levels in a dose-dependent manner (Table 1). The reduction in the blood glucose levels in diabetic rats treated with RHB showed that RHB contains various bioactive compounds with the ability to arrest and reverse oxidative stress-induced destruction of the pancreatic β-islet cells, enhancing β-islet cells regeneration, insulin secretion and consequently the transport of blood glucose to peripheral tissues. Our previous work reported that Ruzu herbal bitters contains some medicinally important phytochemicals such as flavonoids, alkaloids, steroids, phenols, tannins and saponins [13]. There have been reports that the extracts of two component plants of RHB, *Citrullus colocynthis* and *Uvaria chamae*, caused significant decrease in the concentration of blood glucose in alloxan-induced diabetic rats [25], [26], [27]. Our finding is also in agreement with other reports on the antidiabetic activities of polyherbal formulations on alloxan-induced diabetic rats [28], [29]. In contrast to our finding, was a report by Omodamiro *et al.* that "Ruzu herbal bitter which was purchased in Umuahia, caused high blood glucose level in rats" [30]. However, the product we used is "Ruzu herbal bitter**s"** and was purchased from the producer directly from Lagos, Nigeria.

Effect of RHB on the Body Weight

The diabetic-untreated group showed a significant ($p < 0.05$) weight loss throughout the period of the experiment, whereas the normal control group showed a consistent body weight gain (Table 2). Treatment of diabetic rat groups with different doses of RHB caused a significant ($p < 0.05$) body weight gain. Also, the non-diabetic groups treated with RHB showed a consistent weight gain throughout the period of the experiment. Therefore, the result indicated that diabetes causes weight loss, which was countered by RHB. The weight gain observed in the rats treated with RHB showed that the mixture contains essential and non-essential nutrients that can boost the body's nutritional requirements for growth [13]. Similar observation was reported by Otunola and Afolayan who used combined spices for the treatment of diabetes [28]. Also, *Citrullus colocynthis* seeds extract have been reported to cause weight gain in alloxan-induced diabetic rats [26].

Effect of RHB on the Kidney Function Parameters

There were significant ($p < 0.05$) increases in the urea, creatinine, calcium and chloride concentrations with concomitant significant ($p < 0.05$) decreases in the concentrations of sodium, potassium, magnesium and bicarbonate in the diabetic untreated group compared to that of the normal control group (Table 3). However, treatment of diabetic groups with different doses of Ruzu herbal bitters resulted in significant ($p < 0.05$) reductions in the serum concentrations of urea, creatinine, calcium and chloride, with significant ($p < 0.05$) increases in the serum levels of sodium, potassium, magnesium and bicarbonate in a dose-dependent manner. Derangement of water and electrolyte balances may occur in patients with diabetes mellitus, resulting from insulin deficiency, hyperglycemia, and hyperketonemia [31]. Therefore, the reversal of the abnormal levels of urea, creatinine and electrolytes to near normal levels after treating the diabetic rats with RHB revealed that the polyherbal mixture is nephroprotective. Our result is consistent with that reported by previous studies [28], [29], [32]. Also, similar finding was reported by Agarwal *et al.* [25] and

Lakshmi *et al.* [26] who examined the effects of *Citrullus colocynthis* root and seeds extracts respectively on the kidney function parameters of normal and alloxan-induced diabetic rats. Likewise, *Uvaria chamae* root extract caused significant decrease in the concentrations of creatinine and urea in alloxan-induced diabetic rats [27].

In a study on diabetic subjects, Mishra *et al.* reported that serum urea and serum creatinine in diabetic patients were significantly increased with increasing duration of diabetes [33], hence, a conclusion that increase in duration of diabetes was the risk factor for the kidney damage progression [34]. Over time, high blood sugar level causes damage to millions of nephrons, the tiny filtering units in each kidney [35]. Increases in serum creatinine and serum urea occur when there is damage to the kidney or it is not functioning properly. This increase in the blood sugar levels might lead to renal dysfunction. If the kidneys are unable to function normally, the serum creatinine would not be cleared by the kidneys and would increase abnormally [36]. The elevated levels of HbA1c can be lowered by intensive treatment plan, but the elevated levels of serum urea and creatinine which are set on increase due to permanent damage to the kidneys would be difficult to reverse because damage to the kidneys in diabetes mellitus is a permanent phenomenon. Hence, the only way to control this progressive glomerular damage and thereby elevated levels of serum and creatinine web would be early detection and intervention [36].

Hyperglycemia increases serum osmolality, resulting in movement of water out of the cells and subsequently in a reduction of serum sodium levels ($[Na^+]$) by dilution [37]. Diabetes associated hyperkalemia has multiples causes such as reduced glomerular filtration, redistribution of potassium from intracellular to extracellular compartment and alterations in the Na+/K+ ATPase that maintained the transmembrane gradients of sodium and potassium. If the development of diabetes occurred, gastrointestinal and renal losses of potassium lead to hypokalemia. Hypokalemia can result also on insulin administration caused by redistribution of potassium from extracellular to intercellular compartment [37]. Normal potassium concentration is necessary for optimal insulin secretion while depletion can result in reduced glucose tolerance.

Hypomagnesemia can cause hypokalemia possibly because a low intracellular magnesium concentration [Mg^{2+}] activates the renal outer medullary K^+ channel to secrete more K^+ [38]. Hypomagnesemia is a frequent electrolyte disorder in diabetic patients [39]. Osmotic diuresis accompanied by inappropriate magnesiuria is the prominent underlying mechanism of hypomagnesemia in diabetic patients [40]. Except for glucosuria, several other possible explanations for hypomagnesemia in DM have been reported. These include poor dietary intake, glomerular hyperfiltration, altered insulin metabolism, diuretic administration and recurrent metabolic acidosis [39]. Increased gastrointestinal Mg^{2+} losses due to diarrhea as a result of diabetic autonomic neuropathy can also cause low serum Mg^{2+} levels [40]. Elevated intracellular free calcium concentration (by decreasing normal insulin-stimulated glucose transport) increases the requirement for insulin, resulting in hyperparathyroidism-mediated insulin resistance [41].

Effect of RHB on Hematological Indices

There were significant ($p < 0.05$) decreases in the PCV, Hb, WBC and RBC levels, with a significant ($p < 0.05$) increase in the platelets (PLAT) level in the diabetic untreated group compared to the normal control group (Table 4). Treatment of the diabetic groups with different doses of Ruzu herbal bitters resulted in gradual elevations in the PCV, Hb, WBC and RBC levels, with significant reduction in the platelets level in a dose dependent manner when compared to the diabetic-untreated group. It was observed that there were no significant ($p < 0.05$) differences in the RBC counts of all the experimental groups. In general, our result showed almost insignificant differences in the PCV, Hb, PLAT, WBC and RBC levels of the treated groups and that of the normal control. Hence, the result showed that RHB has the capacity of restoring the hematological indices of diabetic rats to near normal levels. Lakshmi *et al.* (2013) reported increased Hb levels in alloxan-induced diabetic rats after treatment with *Citrullus colocynthis* seeds extract. Emordi *et al.* (2018) reported that the root extract of *U. chamae* caused no significant alteration in white blood cell (WBC), red blood cell (RBC), hemoglobin concentration (Hg), packed cell volume (PCV), and platelet (PLT) measurements of the diabetic rats compared to the control.

Hematological indices are important indicators for the evaluation of variations in size, number, and maturity of different blood cells. They are important for the assessment and management of patients with DM [42]. It has been shown that WBC count and PCV have been associated with insulin resistance and T2DM [43]. Positive correlation has been observed between PCV and hyperinsulinemia, high blood pressure, elevated serum triglycerides, low HDL cholesterol, and central obesity, suggesting relationship to insulin resistance [44], [45]. There are some reports that elevated white blood cell count (WBC) is a classical inflammatory marker and is associated with several cardiovascular disease risk factors and diabetes [46], [47]. They could be

activated by advanced glycation end products, angiotensin II, oxidative stress in T2DM, induced by hyperglycemia [48]. However, contrary to some reports on elevated levels of WBC in diabetes [42], [49], in our study, there was a decreased level of WBC in the diabetic rats when compared to the normal control. This decrease in WBC observed in the diabetic rats in our study could be due to immunosuppressive effect caused by diabetes-induced oxidative stress.

Anemia is a common hematological finding in diabetic patients. Anemia is defined as a reduction in the hemoglobin concentration of blood, which consequently reduces the oxygen-carrying capacity of red blood cells such that they are unable to meet the body's physiological needs [49]. Therefore, the reduction in the hemoglobin (Hb) level in the diabetic rats observed in our study could be due to diabetes-induced anemia. However, treatment of the rats with the different doses of RHB increased the Hb level to near normal level. A study has shown that the number of red blood cells is decreased and the lifespan is reduced in T2DM due to elevated blood glucose [50]. This report is in tandem with our study, in which we observed a decreased level of red blood cell (RBC) count in the diabetic rats (although not significantly ($p < 0.05$) different) compared to the normal control rats. Similar report was also given by Milosevic and LukicPanin [51]. It has been reported that low haemoglobin concentration may contribute to complications and progression of diabetes [49]. Low haemoglobin concentration is associated with rapid decline in glomerular filtration rate than that of other kidney diseases [52]. Anemia in patients with diabetes increases susceptibility of the kidney to nephropathy. It is widely accepted that patients with diabetes are more vulnerable to the effects of anemia [53]. Al-Khoury *et al.* observed in their study that for each chronic kidney disease stage, hemoglobin is 1 g/dl lower in patients with diabetes than in the non-diabetic population [54].

Also, we observed increased platelets count in the diabetic rats compared to the normal group. This is in accordance with several studies that have shown an increased number of large circulating platelets in diabetes compared with controls [55], [56], [57]. Patients with type 2 diabetes mellitus (T2DM) have an increased risk of coagulation abnormalities and thromboembolic events. Platelets have a key role and increased adhesion, activation, and aggregation due to dysregulation of several signaling pathways and metabolic disturbances including insulin resistance, hyperglycemia, and dyslipidemia have been noted in diabetic patients [58], [59]. Systematic inflammation, oxidative stress, impaired calcium metabolism, decreased bioavailability of nitric oxide, increased phosphorylation and glycosylation of cellular proteins are responsible for increased platelet activation and increased release of prothrombotic and proinflammatory agents in diabetes [60].

Histological Assessment

Effect of RHB on the Histology of the Kidney

The effect of Ruzu herbal bitters (RHB) on the histology of the kidney of the different groups of alloxan-induced diabetic and non-diabetic rats are presented in Figures 2a and 2b. The normal control rat group (Plate 1) showed the normal renal histomorphological features. The section showed normal glomeruli (G), in bowman's capsules (white arrows) surrounded by numerous renal tubules suspended in a highly vascularised connective tissue meshwork (renal interstitium) and renal tubules (black arrows). In contrast, the cross section of the kidney of the diabetic untreated group (Plate 2) showed a severe degeneration and necrosis of the renal tubules with infiltration of inflammatory mononuclear leukocytes into the renal interstitium. Variably thickening of the Bowman's capsules were observed. Some of the Bowman's space show presence of eosinophillic casts and some of the relatively normal renal tubules showed accumulation of eosinophillic tubular casts inside the lumen. Similar to group 2, the group 4 (Plate 4) treated with 0.14 ml/kg b.w. of RHB showed eosinophillic tubular casts in the renal tubules (white arrows). The tubules in the cortex and medulla were affected. Eosinophillic casts were also observed in the Bowman's spaces (blue arrow). However, groups 5 (Plate 5) and 6 (Plate 6) treated respectively with 0.29 and 0.57 ml/kg b.w. of RHB, including group 3 (Plate 3) treated with glibenclamide showed the normal renal histomorphology similar to the control group. Likewise, normal renal histomorphology was observed in the different non-diabetic groups 7 (Plate 7), 8 (Plate 8) and 9 (Plate 9) treated with 0.14, 0.29 and 0.57 ml/kg b.w. of RHB respectively.

The observation that the diabetic rats treated with 0.29 and 0.57 ml/kg b.w. of RHB showed normal kidney histomorphology is further explained by the reductions in the levels of serum urea and creatinine, including near normal levels of electrolytes and hemoglobin (Hb) which are implicated in renal problem. Hence, the result showed that RHB has nephroprotective effect. This report is in line with other findings [61], [62], [63].

Effect of RHB on the Histology of the Spleen

Shown in figures 3a and 3b, sections of the spleen collected from the animals in all the experimental groups showed the normal splenic histomorphology for laboratory rodents. Each of the sections showed normal white pulp (W) and red pulp (R). The white pulps are made up of predominantly small lymphocytes in the cortical region and large lymphocytes in the medullar areas (Plates 1 - 9). This result is further explained by the result of the red blood cell (RBC) count, which showed no significant ($p < 0.05$) difference in all the experimental groups. Similar results on normal splenic histomorphology for both diabetic untreated and diabetic treated rats have been reported [62], [64], [65]. On the contrary, there were reports that light microscopic examination of sections from the spleen of diabetic rats showed that the white pulp was greatly diffused/increased, while mature lymphocytes in peripheral sections of the spleen were also dramatically reduced in strptozotocin-induced diabetic rats [66], [67].

5 CONCLUSION

The study has shown that diabetes causes kidney damage and hematological changes evidenced by elevated levels of urea and creatinine, electrolyte imbalance, decreased levels of PCV, Hb, WBC, RBC and high level of platelets. However, Ruzu herbal bitters (RHB) was able to reverse these effects to near normal state. Hence, RHB has antidiabetic, nephroprotective and normal hematological effects in the experimental rats. Also, it was found that 0.57 ml/kg b.w. of RHB (equivalent to 40 ml/70 kg body weight daily) is equipotent with 0.5 mg/kg b.w of glibenclamide, a standard antidiabetic drug. Therefore, RHB is recommend in the management of diabetes mellitus. However, further studies should be carried out to ascertain the mechanism of action of Ruzu herbal bitters and its possible side effects.

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