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Research article

Optimum biscuit from *Musa sapientum* L. and *Vigna unguiculata* L. composite flour: effect on pancreatic histology, biochemical and hematological parameters of diabetic rats

Bilkissou Njapndounke [a,**], Marc Bertrand Dandji Saah [a], Marius Edith Foko Kouam [a], Gires Teboukeu Boungo [b], Francois Zambou Ngoufack [a,*]

[a] *Research Unit of Biochemistry, Medicinal Plants, Food Sciences and Nutrition (URBPMAN), Department of Biochemistry, Faculty of Science, University of Dschang, Cameroon*

[b] *Department of Biochemistry, Faculty of Science, University of Bamenda, Cameroon*

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ABSTRACT

This study investigated the effect of consumption of an optimum biscuit from composite flour of *Musa sapientum* L. (*'banana cochon'*) and *Vigna unguiculata* L. (cowpea) on the pancreatic histology, biochemical and hematological parameters of streptozotocin-induced diabetic *Wistar* rats. The optimum biscuit was evaluated for its chemical properties and glycaemic index. The weekly fasting blood glucose level, food intake and weight of the rats were recorded. The effect of 28 days' consumption of different percentages of optimum biscuit with/without Metformin was also evaluated on the pancreatic histology, biochemical and hematological parameters of rats. Results showed that, the optimum biscuit is rich in minerals (potassium, magnesium and calcium), dietary fibre (9.4%) and is a low glycaemic index product (50.91%). Also, the optimum biscuit significantly lowered/maintained the blood glucose level of diabetic rats even though the weekly weights of the rats were reduced while food intake increased. Nonetheless, the hematological parameters of the treated diabetic rats were significantly ($P < 0.05$) improved when compared to the untreated diabetic rats groups. With the exception of total serum protein, other biochemical parameters such as serum creatinine, urea, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase and bilirubin in the treated diabetic groups were significantly ($P < 0.05$) reduced or closer to those of non-diabetic rats. The serum cholesterol, triglyceride, low-density lipoprotein levels were significantly ($p < 0.05$) reduced while the HDL level significantly increased in treated diabetic groups. Histological examination of the pancreas showed that treatment of diabetic groups with optimum biscuit was able to slow down the destruction (protection) of beta-cells. Thus, optimum biscuit could be used to improve the health status during the management and prevention of complications in diabetic patients.

1. Introduction

The change in lifestyle (sedentary) and feeding habits toward the consumption of high energy-containing foods (diets high in sugar and fat) are the major causes of diet-related diseases (Aggarwal et al., 2016a). This has resulted to a high prevalence of non-communicable diseases such as obesity, cardiovascular diseases and diabetes (Aggarwal et al., 2016b). The prevalence of diabetes mellitus and its complication is estimated to be about 8.8% of the world population (Oboh et al., 2020). In recent years, Cameroon is facing an increasing prevalence of diabetes mellitus, from 2.0% in 1999 to 4.7% in 2002 and 5.8% in 2018 (Bigna et al., 2018). Diabetes mellitus is characterized by high blood glucose and altering cellular homeostasis (hypercholesterolemia, high plasma triglycerides and low high-density lipoprotein (HDL) leading to vascular damages (Sone et al., 2016). Drugs are one of the ways used to control the blood plasma, insulin and cholesterol levels of these patients (Bohannon, 2002). Other intervention methods known are physical activity and diet management which is the simplest and cheapest form of managing the disease (Johnson et al., 2013). However, patients with this disease always find it difficult to have food that would provide them with the

* Corresponding author.
** Corresponding author.
E-mail addresses: njapndounkebilkissou123@yahoo.com (B. Njapndounke), fzambou@yahoo.fr (F.Z. Ngoufack).

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necessary energy without changing their blood glucose level (Aggarwal et al., 2016a). Thus, diet control in diabetic patients has led to the intensification of research toward food containing the health-benefiting ingredient(s) and/or food where health-defecting ingredients have been eliminated (Fuentes-Zaragoza et al., 2010). It is in this token that dietary formulations containing easily available, health benefitting and affordable raw materials present in our environment have gain interest. Many studies have been done showing the utilization of *Musa* species in combination with legume and other sources in the formulation of food products. Kapoor et al. (2016) showed that a high fibre diet with reduced calories/fat improved glycaemic response and reduced high serum lipid in patients suffering from diabetes. Olapade et al. (2015) highlighted the use of plantains and cowpea in complementary foods. Also, Sodipo et al. (2020) reported that formulated biscuits from a blend of unripe plantains, moringa seeds and pigeon pea were able to boost the status of streptozotocin-induced rats.

Vigna unguiculata (cowpea) is an available legume known to be widely used than any other legume (Olapade et al., 2015). It is a rich source of protein, resistant starch, dietary fibre, phytochemicals and low fat (Mwangwela, 2006). The latter compositions are at the origin of its protective effect toward several chronic diseases such as gastrointestinal disorder, cardiovascular disease, hypercholesterolemia, obesity and diabetes (Barnes et al., 2015).

Musa sapientum species precisely *'Banane cochon' is* a traditionally known dessert banana grown in Cameroon (Ngoh Newilah et al., 2016). Our previous work showed that this banana has a low protein content (3.10%), but a high dietary fibre (12.99%), potassium (1046.14 mg/100g), calcium (512 mg/100g), phosphorus (89.90 mg/100g), low reducing sugar 0.01 g/100g), and glycaemic index of 28.66%. However, no scientific work to the best of our knowledge has been done to show its transformation in combination with *Vigna unguiculata* (cowpea) into a bakery product and the effects of the latter consumption on streptozotocin-induced diabetic rats. Hence, we need to valorize this new *Musa* species through its transformation to biscuits as it is the most popular bakery product due to its affordability. Thus this work aimed at studying the effect of the consumption of an optimum biscuit from *Musa sapientum* (*'banane cochon'*) and *Vigna unguiculata* (cowpea) flour on the pancreatic histology, biochemical and hematological parameters of streptozotocin-induced diabetic *Wistar* rats.

2. Materials and methods

2.1. Biological materials, chemicals and ingredients

Bunches of *Musa sapientum* (*'banane cochon'*) were harvested in a banana plantation in Fossong-Wentcheng village (Menoua division, West region, Cameroon) in May 2020. The sample was authenticated under voucher number 34046 National Herbarium of Cameroun (NHC). *Vigna unguiculata* (Variety Br 12), milk, sweetener, salt, egg and margarine (shortening) were bought in the local market of the Dschang subdivision (Menoua division West region, Cameroon).

2.2. Production of Musa sapientum ('banane cochon') and Vigna unguiculata (cowpea) flour

The method proposed by Tchango and Ngalani (1998) with slight modification was used for the production of the banana flour. Here, undamaged unripe green bunches of *banane cochon* at stage 1 were washed using potable water. They were then peeled and pulps cut into cubes of about 1 cm3, resulting in "cossettes" which underwent boiling for 5 min in a water bath at a temperature of 75 °C. The drying was carried out for 48 h in a Venticell electric oven (BMT Medical Technology s.r.o., Cejl 157/50, Zábrdovice, 602 00 Brno, Czech Republic) whose temperature was controlled at 45 °C. The dried banana pulp was ground using an ordinary artisanal mill. Regarding the production of cowpea flour, the protocol proposed by Olapade et al. (2015) was used. This method consists of cleaning and soaking of cowpea for a period of 12h in potable water to remove flocculent agent, the grains were later dehulled and dried in an oven (Venticell type) at a temperature of 45 °C for 48h. The dry dehulled grains were grown and sieved to obtain cowpea flour.

2.3. Formulation of optimum biscuit from composite flour of Musa sapientum ('banane cochon') and Vigna unguiculata

The optimum combination of *Musa sapientum* (*'banane cochon'*) and *Vigna unguiculata* flour was determined using an augmented simplex lattice mixture design. This design generated different formulations of flour samples and the optimum composition was based on the reference lipid content of 18.75%, the protein content of 10.30% and a reducing sugar lower than 20.14% (Erukainure et al., 2013a, 2013b). Thus the proportion was as thus *Musa sapientum* (*'banane cochon'*) 67.5g and *Vigna unguiculata* (cowpea) 17.5g, for a total mass of 85g of flour. The quantities of non-variable parameters such as sweetener, milk, yeast, the volume of potable water, egg, salt, baking temperature and time respectively were as thus: 0.375 g, 3.8 g, 6 g, 15.65 ml, 55 g (with shell), 0.13 g, 150 °C and 26 min. They were fixed based on preliminary tests and previous work (Teshome et al., 2017). The creaming method of mixing proposed by Manohar and Haridas (1997) was used for the production of biscuits. Here, sweetener (saccharin and cyclamate) and margarine were creamed in an electric mixer (with a mixing degree from 1 to 5) with a flat beater for 2 min at the mixing degree of 3. Dough water containing baking chemicals and sodium chloride was added to the cream and mixed for 5 min at 125 rpm to obtain a homogeneous cream. Finally, flour sieved twice with baking powder was added and mixed for 3 min at 61 rpm. The dough was later cut using a biscuit cutter and baked at 150 °C for 26 min to obtain the optimum biscuit.

2.4. Determination of nutritional value, calorific value and glycaemic index of optimum biscuit

The optimum biscuit from composite flour of *Musa sapientum* (*'banane cochon'*) and *Vigna unguiculata* was subjected to analyses. The methods proposed by AOAC (1990) were used to evaluate the moisture content, dry matter content, carbohydrate and caloric value, crude fibre, lipid, protein, ash content, as well as the K, Na, Ca, Mg, P, Fe, Zn and Cu. Saponin was determined using the method proposed by Koziol (1990). The method proposed by Reddy et al. (1999) was used to evaluate the phytate content. Day and Underwood (1986) method was used to evaluate the oxalate content. For the evaluation of glycaemic index, 10 healthy, (5 males and 5 females) volunteers aged between 27-40years, with a body mass index of less than 25 kg/m^2; non-smokers, not pregnant nor diabetic and none of them had a family history of diabetes were recruited. The protocol of this study was approved by the Ethical Committee of the Ministry of Public Health of Cameroon under the number No 2020/05/60CE/CNERH/SP. All the individuals signed a consent form before their inclusion in the study. The subjects were given full details of the study protocol and had the opportunity to ask questions before the study. The subjects were asked not to undertake vigorous activities the day before the test and to avoid caffeine-containing drinks 24 h before the test. Also, a testing session after a 10–12 h overnight fast on the day test was performed. On the first day, subjects were given the standard or reference carbohydrate: 50 g of glucose dissolved in 150 ml of drinkable water. Blood glucose level was measured in capillary whole blood obtained by finger prick by a nurse and placed in a glucometer (On-Call

Sharp, ACON lab, inc San Diego, USA) in the fasted state which constitutes for time 0 and after 30, 60, 90 and 120 min on the consumption of the reference (glucose) food. Blood glucose curves were constructed and the incremental area under the curve (IAUC) was calculated for reference food (glucose) by the trapezoidal rule (Wolever and Jenkins, 1986). A similar procedure was repeated with test food (biscuit) but in this case, 87.19 g of biscuit was consumed since the available carbohydrate content was 57.34 g/100g.

2.5. Effect of consumption of optimum biscuit on diabetic and non-diabetic Wistar rats

2.5.1. Animal experimentation

In this study, 36-seven-week-old male and female *Wistar* rats weighing 150g–200g were used and housed in polycarbonate cages. In each group, an equal number of females and males was used and the mean was taken as this disease affects both males and females. These rats were obtained from the crossed parent and reared by us at the Biochemistry animal house of the University of Dschang. They were maintained at room temperature of 20 ± 2 °C with a 12h light per 12h dark cycle. They had access to diets and water ad-libitum. All the animal experiments were approved by the Local Ethical Committee of the Faculty of Science (University of Dschang-Cameroon) and were designed following the internationally accepted standard ethical guidelines for laboratory animals' use and care as described in the guidelines of the European Union Institutional Ethics committee on Animal Care (Council EEC 86/609/EEC of the 24th November 1986). This section report adhere to the Guidelines for reporting animal research (Kilkenny et al., 2010). The rats were acclimatised for one week and the test treatment lasted for four weeks. Streptozotocin (STZ) was procured from Sigma-Aldrich (Sigma-Aldrich chemie Gmbh, Eschenstr, Taufkirchen, Germany), Metformin and all reagents used were analytically upgraded.

2.5.2. Preparation of streptozotocin and induction of diabetes

The streptozotocin (STZ) (Sigma –Aldrich chemie Gmbh, Eschenstr, Taufkirchen, Germany) was dissolved in a freshly prepared citrate buffer (0.1 M, pH 4.5). A single dose of STZ at 55 mg/kg was administered intraperitoneal with the aid of an insulin syringe to overnight fasted rats while the non-diabetic group received citrate buffer (0.1 M) (Chaudhary and Mudgal, 2020). The induced groups were immediately given glucose 5% to prevent hypoglycemia. After 48 h (two days), rats with blood glucose levels more or equal 250 mg/dl were considered to be diabetes (Aggarwal et al., 2016a). Also, feeding of rats with the test biscuit started immediately that day. It has to be noted that, we had 24 diabetic rats and 12 normal rats.

2.5.3. Experimental design and diet proportions

The animals were divided randomly into nine groups of four rats each. The rats were fed a total of 40 g of diet per day (Chaudhary and Mudgal, 2020). The chosen percentage of substitution used in this study was based on the work of Silva et al. (2016); where they show that green banana paste diet at a concentration of 75% and 50% prevents hyperglycemia, high cholesterol, oxidative damage in the liver and kidney. Also, Metformin was administered at the dose of 40 mg/kg/day lower than 50 mg/kg/day used by Silva et al. (2016) to see the capacity of our formulated optimum biscuit to ameliorate the activity Metformin. The groups were as follows:

Group 1: Non-diabetic rats fed with 100% standard diet.

Group 2: Non-diabetic rats fed with 50% standard diet and 50% optimum biscuit.

Group 3: Non-diabetic rats fed with 25% standard diet and 75% optimum biscuit.

Group 4: Diabetic rats fed with 100% standard diet.

Group 5: Diabetic rats fed with 50% standard diet and 50% optimum biscuit.

Group 6: Diabetic rats fed with 25% standard diet and 75% optimum biscuit.

Group 7: Diabetic rats fed with 100% standard diet and Metformin at 40 mg/kg.

Group 8: Diabetic rats fed with 50% standard diet and 50% optimum biscuit and Metformin at 40 mg/kg.

Group 9: Diabetic rats fed with 25% standard diet, and 75% optimum biscuit and Metformin at 40 mg/kg.

The composition of the standard diet was as thus: ground whole corn, fish meal, soybean, salt, vegetable oil, vitamin premix, bone meal (Telefo, 1998). During the treatment, the weekly weight, food intake and blood glucose were evaluated. The measurement of these parameters started at Day 0 which was the day before streptozotocin was administered and Day 1 was the day the streptozotocin-diabetic groups were confirmed with a blood glucose of more or equal to 250 mg/dl.

2.5.4. Euthanasia, collection of blood samples, and preparation of serum

At the end of the four weeks treatment, the animals fasted for 12h and anesthesia. Blood was collected by cardiac puncture and introduced in dry tubes and EDTA tubes. After 3h, the fresh blood in dry tubes was centrifuged at 3500 rpm for 15 min. Serum was obtained and conserved at -18 °C for further analyses.

2.5.5. Assessment of serum biochemical parameters

Here, we studied the following parameters total protein, creatinine, alanine aminotransferase (ALAT), alkaline phosphatase (ALP) and aspartate aminotransferase (ASAT). Also, triglyceride (TG), total cholesterol (TC), bilirubin (BIL) and high-density lipoprotein (HDL) were evaluated. Handling of the latter parameters was done following the protocol of the kit manufacturer. The direct determination method was used for low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) levels as proposed by Friedewald et al. (1972). The atherosclerotic index was calculated from the following formula (Eq. (1)) proposed by Friedewald et al. (1972).

$$\text{Atherosclerostic index} = \frac{(\text{VLDL cholesterol} + \text{LDLcholesterol})}{\text{HDLcholesterol}} \quad (1)$$

2.5.6. Evaluation of hematological parameters

Freshly collected blood samples in EDTA tubes were used and with the aid of an automatic coulter (*Shenzhen Mindray BC-3600, Chine*), blood cells were counted. The hematological parameters analysed included white blood cells (WBCs), lymphocytes and granulocytes; red blood cells (RBCs), hemoglobin (Hb), mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT) and Hematocrit (HCT).

2.5.7. Histopathological analysis of the pancreas

After the animals were euthanized, the pancreas was collected and weighed. The pancreas was washed in a 0.9% NaCl solution and immediately preserved in 10% formaldehyde. The histological analyses were done following the method proposed by Di-Fiore (1963). Briefly, after dehydration with alcohol; using a microtome (ACCU-Cut SRM), five μm thick cuts were made on the organ and stained with hematoxylin and eosin. These colored sections were covered with a thin glass plate and observed under an optical microscope (Olympus BX51) equipped with a camera (400X) for taking photographs. A comparative histopathological study of the pancreas was performed.

2.5.8. Analysis of data

Data of chemical, glycaemic index and animal parameters expressed as mean ± SD were statistically analysed using one-way analysis of variance (ANOVA). Waller Duncan's post hoc test was used to compare the significance between the means values using the IBM SPSS statistic 22 (*Chicago, IL, USA*). A p-value < 0.05 was considered statistically significant.

Table 1. Nutritional composition, caloric value and glycaemic index of optimum biscuit.

Parameters	Optimum biscuit
Energy (Kcal/100 g)	472.85 ± 0.02
Protein (%)	9.64 ± 0.20
Lipid content (%)	18.73 ± 0.20
Moisture content (%)	2.06 ± 0.02
Dry matter content (%)	97.95 ± 0.02
Ash content (%)	5.20 ± 0.28
Total carbohydrate (%)	66.74 ± 0.02
Dietary fibre (%)	9.40 ± 0.42
Calcium (mg/100 g)	1207.50 ± 10.60
Magnesium (mg/100 g)	321.80 ± 0.81
Phosphorous (mg/100 g)	690.66 ± 0.89
Copper (mg/100 g)	0.20 ± 0.00
Sodium (mg/100 g)	2.14 ± 0.00
Potassium (mg/100 g)	693.36 ± 0.00
Iron (mg/100 g)	11.55 ± 0.05
Zinc (mg/100 g)	0.65 ± 0.01
Reducing sugar (%)	12.33 ± 0.30
Oxalate (mg/100 g)	9.675 ± 0.31
Phytate (mg/100 g)	6.08 ± 0.45
saponin (mg/100 g)	2.64 ± 0.00
Glycaemic index	50.91 ± 0.01

Values are mean ± SD.

3. Results and discussion

3.1. Nutritional value, energy value and glycaemic index of optimum biscuit from composite flour of Musa sapientum ('banane cochon') and Vigna unguiculata

Table 1 depicts the nutritional composition, glycaemic index and energy value of the optimum biscuit from composite flour of *Musa sapientum* ('*banane cochon*') and *Vigna unguiculata*. We can infer from this table that, this biscuit contains potassium, calcium, iron, magnesium, phosphorus, copper, sodium, zinc, low moisture content, dietary fibre, oxalate, saponin and phytate. Also, it is a low glycaemic index product, with a low reducing sugar and recommended protein, lipid content and energy value. The presence of minerals such as calcium, magnesium, phosphorus, potassium, zinc, sodium and iron in the formulated biscuit even though the bioavailability was not evaluated shows that this biscuit could be a good source of minerals. Erukainure et al. (2015) found that the presence of these minerals in the formulated diet can stabilise the hepatic plasma membrane and repair hepatic tissues. Also, the low moisture content of this product is of great benefit as it would be less prone to microbial attack and thus can be accessed by rural communities that lack basic storage facilities and electricity. Similar moisture content, lipid content and protein were obtained by Erukainure et al. (2013a). The high fibre content of this product is of great interest as it provides medical properties. Studies have provided evidence of the hypoglycaemic properties of fibre-containing food (Erukainure et al., 2013c; Ajiboye et al., 2018). Concerning phytochemicals, their antidiabetic effect has been reported by studies done by Xi et al. (2008). The low reducing sugar content of this biscuit is due to the low sugar-containing ingredients (unripe *banane cochon* and cowpea). According to Reyes-Perez et al. (2013), the glycaemic index of food determines its potential in increasing or decreasing postprandial blood glucose levels. The glycaemic index of the formulated biscuit (50.91) is in the range of 0–55 which is known for low glycaemic product (Huang and Miskelly 2017).

3.2. Changes in biochemical, hematological and pancreatic histology of diabetic and non-diabetic Wistar rats upon consumption of optimum biscuit

3.2.1. Effect of consumption of optimum biscuit on fasting blood glucose level of non-diabetic and diabetic rats

Table 2 shows the fasting blood glucose level of rats fed for 28 days. This table reveals that, before the induction of diabetes (Day 0), rats of all groups had normal fasting blood glucose. The latter parameter increased upon induction with streptozotocin which was confirmed with fasting blood glucose of more or equal to 250 mg/dl 48 h after (Day 1 of treatment with biscuit). Also, normal rats taking standard diets (groups 1) as well as those taking 50% and 75% biscuit (groups 2 and 3) had no significant variation in their fasting blood glucose level during the test period. Non-treated streptozotocin-induced rats (group 4) on the other hand, had a significant ($P < 0.05$) increase in fasting blood glucose level when compared with non-diabetic groups (group 1, 2 and 3) and diabetic groups fed on the biscuit and/or Metformin (groups 5, 6, 7, 8 and 9). Also, diabetic rats taking 50%, 75% substituted diet and taking Metformin (groups 8 and 9) had significantly ($P < 0.05$) decreased fasting blood glucose compared to those of groups 5, 6 and 7. Rats taking 75% biscuit (group 9) had the lowest decreasing fasting blood glucose level compared to others. The increasing prevalence of diabetes and its complications, mostly in developing countries, besides with high treatment cost has led to the search, development of novel and cheap treatment for the disease (Erukainure et al., 2013c). Adequate caloric intake, maintenance of normal blood glucose level and intake of food rich in dietary fibres are some means used to managed and/or treat this disease (Aggarwal et al., 2016a). Induction of diabetes results in to increase in fasting blood glucose but upon feeding on a diet substituted with the optimum biscuit, the fasting blood glucose level reduced/maintained since this biscuit has

Table 2. Fasting blood glucose level of diabetic and non-diabetic rats fed for 28 days.

Day	group 1	group 2	group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
0	76.00 ± 2.16^{b}	76.00 ± 2.94^{b}	73.75 ± 4.35^{b}	74.00 ± 2.94^{b}	76.20 ± 3.42^{b}	68.00 ± 1.41^{a}	73.60 ± 2.07^{b}	73.83 ± 2.14^{b}	75.25 ± 0.50^{b}
1	75.50 ± 5.75^{a}	70.75 ± 2.22^{a}	73.25 ± 4.43^{a}	317.5 ± 2.89^{b}	337.80 ± 10.38^{c}	343.80 ± 16.16^{d}	414.80 ± 13.74^{e}	428.33 ± 21.86^{f}	413.25 ± 1.71^{e}
7	75.50 ± 3.11^{a}	76.00 ± 1.83^{a}	83.75 ± 5.56^{a}	418.75 ± 2.50^{f}	275.00 ± 2.65^{b}	284.4 ± 13.09^{c}	337.80 ± 12.46^{d}	373.83 ± 12.22^{e}	373.50 ± 11.36^{e}
14	70.75 ± 1.50^{a}	74.50 ± 3.32^{a}	75.25 ± 3.78^{a}	449.5 ± 7.33^{e}	259.80 ± 3.63^{b}	259.20 ± 10.38^{b}	327.60 ± 3.72^{d}	292.83 ± 3.71^{c}	256.25 ± 4.50^{b}
21	76.25 ± 1.89^{a}	69.25 ± 6.13^{a}	73.5 ± 2.89^{a}	450.5 ± 1.00^{f}	252.80 ± 7.63^{d}	246.20 ± 11.90^{b}	279.80 ± 17.85^{e}	239.67 ± 13.06^{b}	249.25 ± 2.06^{c}
28	76.25 ± 1.89^{a}	69.75 ± 3.30^{a}	73.5 ± 4.36^{a}	527.25 ± 6.65^{f}	234.80 ± 14.33^{d}	234.00 ± 4.12^{d}	246.60 ± 10.11^{e}	227.83 ± 12.51^{c}	213.25 ± 1.50^{b}

Values (mean ± standard deviation, n = 4) with different superscript letters in the same row are significantly different at p<0.05, group 1: Non diabetic rats fed with 100% standard diet only, group 2: Non diabetic rats fed with 50% standard diet and 50% biscuit, group 3: Non diabetic rats fed with 25% standard diet and 75 % biscuit, group 4: Diabetic rats fed with 100% standard diet only, group 5: Diabetic rats fed with 50% standard diet and 50% biscuit group 6: Diabetic rats fed with 25% standard diet and 75% biscuit, group 7: Diabetic rats fed with 100% standard diet and Metformin at 40 mg/kg, group 8: Diabetic rats fed with 50% standard diet, and 50% biscuit and Metformin at 40 mg/kg, group 9: Diabetic rats fed with 25% standard diet and 75% biscuit and Metformin at 40 mg/kg, Day 0 = day before the induction of diabetes, Day 1 = 48 h after induction and confirmation of fasting blood glucose of more or equal 250 mg/dl.

Table 3. Weight of diabetic and non-diabetic rats fed for 28 days.

Day	group 1	group 2	group 3	group 4	group 5	group 6	group 7	group 8	group 9
0	192.25 ± 4.57^{f}	145.75 ± 2.50^{b}	154.25 ± 2.63^{cd}	158.25 ± 4.72^{d}	148.20 ± 6.42^{bc}	133.60 ± 2.07^{a}	135.60 ± 3.21^{a}	178.17 ± 6.49^{e}	156.75 ± 5.85^{d}
1	199.00 ± 0.82^{f}	147.25 ± 0.96^{d}	160.75 ± 7.37^{e}	132.00 ± 5.35^{bc}	136.40 ± 2.30^{c}	126.60 ± 3.91^{b}	117.60 ± 1.14^{a}	148.50 ± 3.08^{d}	135.25 ± 6.08^{c}
7	215.25 ± 5.19^{g}	164.75 ± 2.63^{e}	180.00 ± 5.60^{f}	116.00 ± 2.16^{b}	124.40 ± 4.45^{c}	124.20 ± 2.77^{c}	107.60 ± 5.03^{a}	136.00 ± 1.79^{d}	125.25 ± 2.99^{c}
14	228.50 ± 2.08^{f}	178.00 ± 3.27^{d}	187.00 ± 6.38^{e}	104.75 ± 1.71^{a}	123.80 ± 1.10^{c}	114.20 ± 1.92^{b}	105.20 ± 5.36^{a}	124.67 ± 3.20^{c}	122.75 ± 2.99^{c}
21	236.75 ± 2.75^{e}	185.25 ± 4.11^{d}	188.25 ± 2.63^{d}	101.25 ± 2.50^{a}	122.60 ± 1.67^{c}	113.00 ± 1.87^{b}	101.00 ± 3.08^{a}	122.50 ± 2.59^{c}	120.50 ± 1.91^{c}
28	242.50 ± 2.65^{f}	185.00 ± 4.24^{d}	202.75 ± 3.30^{e}	95.75 ± 4.03^{a}	122.20 ± 1.48^{c}	112.00 ± 1.00^{b}	99.40 ± 1.34^{a}	118.50 ± 3.62^{c}	119.25 ± 1.71^{c}

Values (mean ± standard deviation, n = 4) with different superscript letters in the same row are significantly different at $p<0.05$, group 1: Non diabetic rats fed with 100% standard diet only, group 2: Non diabetic rats fed with 50% standard diet and 50% biscuit, group 3: Non diabetic rats fed with 25% standard diet and 75 % biscuit, group 4: Diabetic rats fed with 100% standard diet only, group 5: Diabetic rats fed with 50% standard diet and 50% biscuit group 6: Diabetic rats fed with 25% standard diet and 75% biscuit, group 7: Diabetic rats fed with 100% standard diet and Metformin at 40 mg/kg, group 8: Diabetic rats fed with 50% standard diet, and 50% biscuit and Metformin at 40 mg/kg, group 9: Diabetic rats fed with 25% standard diet and 75% biscuit and Metformin at 40 mg/kg, Day 0 = day before the induction of diabetes, Day 1 = 48 h after induction and confirmation of fasting blood glucose of more or equal 250 mg/dl.

a low reducing sugar content and high dietary fibre. Foods containing dietary fibres are reported to slow down gastric emptying by coating the small intestine and slowing down the uptake of glucose as it reduces the breakdown of glucose (Fuentes-Zaragoza et al., 2010). Similar results were obtained by Erukainure et al. (2013a), where fibre-enriched biscuit led to a significant reduction in fasting blood glucose levels. The high percentage substitution of diet in group 9 of 75% and Metformin usage might be at the origin of the lowest reducing blood glucose level in this group. This observation is similar to that of Silva et al. (2016), where 75% of banana pasta prevents complications associated with diabetes since hyperglycemia in diabetic rats was avoided.

3.2.2. *Effect of consumption of optimum biscuit on the weight of non-diabetic and diabetic rats*

The changes in the weight of diabetic and non-diabetic rat depicted in Table 3 show a significant ($P < 0.05$) difference between the weights of non-diabetic and diabetic groups. Also, non-diabetic rats (groups 1, 2 and 3), experienced an increasing weight while the diabetic rats (groups 4, 5, 6, 7, 8 and 9) had a decreasing weight with increasing feeding days. According to Heap et al. (2004), diabetes is linked with bone mass reduction. This could be responsible for the observed drastic reduction in weight of non-treated diabetic rats. In this study, the significant reduction in weight in diabetic rats can be explained by the breakdown of protein and fats which causes degeneration of adipose cells and muscle tissues which is characteristic of diabetes mellitus (Esonu et al., 2001). Since diabetes is a metabolic disorder, it impacts the bioavailability of nutrients and thus interfering with growth and development. The increasing weight in the non-diabetic groups is due to the presence of insulin used for the regulation of blood glucose level resulting to increase bone formation, the nutrient content of the biscuit and thus weight increased (Jiao et al., 2015). Similar observations were obtained by Chaudhary and Mudgal (2020), where they show that finger millet (Eleusine coracana) enriched probiotic fermented milk had an antidiabetic and hypolipidemic effect.

3.2.3. *Effect of consumption of optimum biscuit on food intake of non-diabetic and diabetic rats*

Table 4 shows the mean food intake for each rat in the diabetic and non-diabetic groups during the experimental period. We can depict that apart from day zero (day 0), there was a general significant difference in the food intake of the non-diabetic groups, treated diabetic and non-treated diabetic groups. Also, the disease states of rats resulted in to decrease in food intake noticed in the diabetic groups during day 7. Nonetheless, after this day, diabetic groups experienced an increase in food intake during the experimental period with group 4 having a significant increase compared to treated diabetic groups. The high insulin resistance and poor glycaemic control could be the origin of increased food intake with the resulting decrease in body weight (Cordero-Herrera et al., 2015). Similar observations were obtained by Ma and Mu (2016) where the anti-diabetic effects of soluble and insoluble dietary fibre from deoiled cumin in low-dose streptozotocin and high glucose-fat diet-induced type 2 diabetic rats was studied.

3.2.4. *Effect of consumption of optimum biscuit on serum biochemical parameters of diabetic and non-diabetic rats*

The changes in serum lipid profile parameters over the experimental period are shown in Table 5. It has to be noted that, the diabetic group taking standard diet (group 4) experienced a significant highest ($P < 0.05$) value of triglyceride, total cholesterol, LDL, atherosclerotic index, VLDL and significantly lowest HDL when compared to those of the non-diabetic groups (1, 2 and 3) and treated diabetic groups (5, 6, 7, 8, and 9). Among non-diabetic groups, apart from total cholesterol, there was a significant difference in the lipid profile parameters of group 1, 2 and 3 with group 3 having the lowest TG. The normal health state (good

Table 4. Food intake (g/day) of diabetic and non-diabetic rats fed for 28 days.

Day	group 1	group 2	group 3	group 4	group 5	group 6	group 7	group 8	group 9
0	36.75 ± 0.50^{b}	36.38 ± 0.48^{b}	36.43 ± 0.49^{b}	33.83 ± 0.56^{a}	35.54 ± 0.45^{a}	36.30 ± 0.45^{b}	36.66 ± 0.61^{b}	36.32 ± 0.49^{b}	36.25 ± 0.50^{b}
1	36.75 ± 0.50^{c}	36.38 ± 0.48^{bc}	36.43 ± 0.49^{bc}	33.75 ± 0.50^{a}	35.28 ± 0.43^{a}	35.94 ± 0.41^{b}	36.42 ± 0.63^{bc}	35.88 ± 0.22^{ab}	35.78 ± 0.17^{ab}
7	36.80 ± 0.78^{d}	36.30 ± 0.36^{d}	36.45 ± 0.53^{d}	29.25 ± 0.96^{a}	34.12 ± 0.22^{c}	34.02 ± 0.04^{bc}	33.30 ± 0.40^{b}	33.45 ± 0.45^{b}	33.33 ± 0.46^{b}
14	37.43 ± 0.33^{d}	37.33 ± 0.47^{cd}	36.85 ± 0.87^{cd}	36.03 ± 0.05^{a}	36.68 ± 0.33^{bc}	36.68 ± 0.33^{bc}	36.68 ± 0.33^{bc}	36.25 ± 0.05^{ab}	36.20 ± 0.00^{ab}
21	37.85 ± 0.10^{e}	37.50 ± 0.58^{de}	36.85 ± 0.87^{bc}	37.25 ± 0.50^{cd}	36.36 ± 0.22^{ab}	36.72 ± 0.18^{ab}	36.36 ± 0.22^{ab}	36.25 ± 0.05^{a}	36.20 ± 0.00^{a}
28	37.68 ± 0.15^{f}	37.53 ± 0.55^{e}	37.08 ± 0.10^{d}	37.72 ± 0.19^{f}	36.60 ± 0.16^{ab}	36.88 ± 0.13^{bc}	37.53 ± 0.55^{f}	36.37 ± 0.05^{a}	36.30 ± 0.12^{a}

Values (mean ± standard deviation, n = 4) with different superscript letters in the same row are significantly different at $p<0.05$, group 1: Non diabetic rats fed with 100% standard diet only, group 2: Non diabetic rats fed with 50% standard diet and 50% biscuit, group 3: Non diabetic rats fed with 25% standard diet and 75 % biscuit, group 4: Diabetic rats fed with 100% standard diet only, group 5: Diabetic rats fed with 50% standard diet and 50% biscuit group 6: Diabetic rats fed with 25% standard diet and 75% biscuit, group 7: Diabetic rats fed with 100% standard diet and Metformin at 40 mg/kg, group 8: Diabetic rats fed with 50% standard diet, and 50% biscuit and Metformin at 40 mg/kg, group 9: Diabetic rats fed with 25% standard diet and 75% biscuit and Metformin at 40 mg/kg, Day 0 = day before the induction of diabetes, Day 1 = 48 h after induction and confirmation of fasting blood glucose of more or equal 250 mg/dl.

Table 5. Effect of consumption of optimum biscuit on serum biochemical parameters of diabetic and non-diabetic rats.

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
TG (mg/dL)	177.95 ± 2.12^{c}	146.64 ± 0.68^{b}	93.40 ± 2.31^{a}	318.75 ± 1.36^{h}	236.60 ± 4.94^{d}	250.47 ± 2.83^{f}	259.67 ± 3.37^{g}	245.52 ± 1.48^{e}	175.47 ± 1.41^{c}
TC (mg/dL)	101.16 ± 1.06^{ab}	102.84 ± 3.56^{ab}	110.40 ± 9.41^{bc}	365.04 ± 3.56^{e}	99.84 ± 0.67^{a}	113.38 ± 4.78^{cd}	122.40 ± 6.37^{d}	108.88 ± 10.06^{abc}	115.68 ± 7.07^{cd}
HDL (mg/dL)	47.04 ± 3.11^{f}	41.04 ± 0.83^{e}	34.32 ± 1.27^{c}	21.48 ± 0.60^{a}	34.66 ± 2.08^{c}	32.16 ± 1.48^{bc}	34.44 ± 2.55^{c}	30.72 ± 1.14^{b}	37.60 ± 0.28^{d}
LDL (mg/dL)	18.53 ± 3.42^{a}	32.47 ± 2.77^{b}	57.40 ± 10.07^{d}	279.81 ± 3.23^{e}	17.86 ± 2.67^{a}	31.12 ± 6.02^{b}	36.03 ± 5.44^{b}	29.06 ± 9.89^{b}	48.27 ± 4.47^{c}
Atherosclerotic index	1.160 ± 0.14^{a}	1.51 ± 0.05^{b}	2.23 ± 0.37^{c}	16.00 ± 0.44^{e}	1.89 ± 0.18^{c}	2.53 ± 0.25^{d}	2.56 ± 0.21^{d}	2.55 ± 0.31^{d}	2.59 ± 0.19^{d}
VLDL (mg/dL)	35.59 ± 0.43^{c}	29.33 ± 0.14^{b}	18.68 ± 0.46^{a}	63.75 ± 0.27^{h}	47.32 ± 0.99^{d}	50.09 ± 0.57^{f}	51.93 ± 0.67^{g}	49.10 ± 0.29^{e}	35.09 ± 0.28^{c}
ALP (IU/L)	97.76 ± 0.82^{a}	98.59 ± 0.82^{a}	97.35 ± 2.33^{a}	246.26 ± 2.82^{f}	136.62 ± 2.15^{d}	132.66 ± 1.88^{c}	165.83 ± 0.95^{e}	131.45 ± 0.85^{c}	127.60 ± 0.95^{b}
BIL (mg/dl)	3.68 ± 0.13^{ab}	3.23 ± 0.68^{ab}	2.71 ± 0.80^{a}	11.61 ± 0.30^{d}	5.57 ± 1.31^{c}	4.13 ± 0.49^{b}	5.48 ± 0.39^{c}	3.61 ± 1.21^{ab}	3.10 ± 0.26^{ab}
ALAT (IU/L)	22.06 ± 3.64^{ab}	22.26 ± 2.34^{ab}	20.23 ± 3.30^{a}	100.16 ± 6.91^{c}	25.09 ± 1.81^{ab}	24.28 ± 0.00^{ab}	26.30 ± 2.34^{b}	26.30 ± 2.22^{b}	20.23 ± 4.05^{a}
ASAT (IU/L)	45.94 ± 0.00^{a}	48.81 ± 5.74^{a}	51.68 ± 6.63^{a}	101.06 ± 4.59^{e}	78.09 ± 5.14^{c}	78.09 ± 5.14^{c}	86.13 ± 6.63^{d}	68.88 ± 0.05^{b}	72.73 ± 6.63^{bc}
Total protein (g/dl)	6.05 ± 0.02^{b}	6.56 ± 0.52^{cd}	6.24 ± 0.23^{bc}	3.46 ± 0.15^{a}	6.17 ± 0.15^{bc}	6.22 ± 0.10^{bc}	6.07 ± 0.20^{b}	6.18 ± 0.32^{bc}	6.62 ± 0.27^{d}
Creatinine (mg/dl)	2.52 ± 0.17^{a}	3.44 ± 0.23^{bc}	3.57 ± 0.30^{bc}	6.31 ± 0.42^{e}	3.71 ± 0.12^{d}	3.85 ± 0.29^{d}	3.49 ± 0.17^{bc}	3.24 ± 0.29^{b}	2.53 ± 0.27^{a}
Urea (mg/dl)	90.00 ± 0.00^{a}	105.00 ± 17.32^{ab}	97.50 ± 15.00^{a}	352.50 ± 15.00^{e}	189.00 ± 13.42^{d}	118.20 ± 4.02^{bc}	202.50 ± 15.00^{d}	135.00 ± 16.43^{c}	89.00 ± 1.73^{a}

Values (mean ± standard deviation, n = 4) with different superscript letters in the same row are significantly different at $p<0.05$. group 1: Non diabetic rats fed with 100% standard diet only, group 2: Non diabetic rats fed with 50% standard diet and 50% biscuit, group 3: Non diabetic rats fed with 25% standard diet and 75 % biscuit, group 4: Diabetic rats fed with 100% standard diet only, group 5: Diabetic rats fed with 50% standard diet and 50% biscuit group 6: Diabetic rats fed with 25% standard diet and 75% biscuit, group 7: Diabetic rats fed with 100% standard diet and Metformin at 40 mg/kg, group 8: Diabetic rats fed with 50% standard diet, and 50% biscuit and Metformin at 40 mg/kg, group 9: Diabetic rats fed with 25% standard diet and 75% biscuit and Metformin at 40 mg/kg.

metabolism) of group 1 and 2 could be the reason for no change in TC. The high percentage substitution (75%) in group 3 might be at the origin of the low TG value. It has to be noted that group 9 had the highest HDL level compared to other treated diabetic groups. According to Sone et al. (2016), diabetes is characterized by hyperglycemia and dyslipidemia which in turn is manifested by increase triglycerides, total cholesterol, LDL VLDL and decrease HDL. From a general point of view, our study had shown that feeding on optimum biscuit decrease triglycerides, total cholesterol, LDL, VLDL, atherosclerosis index and increases HDL when treated diabetic rats are compared to untreated diabetic rats. This reduction could be the antilipidaemic effect of the biscuit. The possible explanation is the high fibre content of the biscuit. Fibres are known to be hypolipidaemic as it can lower cholesterol level since it provides a substrate for the production of short-chain fatty acids such as propionate and butyrate. These short fatty acids can counteract the induction of liver cholesterol synthesis which in turn causes increase excretion of bile acids (Weitkunat et al., 2015). Also, during the production of biscuits, we use margarine (shortening) characterized with low trans, low inter-esterified fatty acids and high polyunsaturated fatty acids content. Makni et al. (2011), has shown that polyunsaturated fatty acids had a strong hypotriglyceridaemic and hypocholesterolemic impact on rats with a reduction in LDL and increase HDL. The results obtain in this study is in the same token with that of Erukainure et al. (2013a), who reported that feeding on fibre-enriched biscuit led to a significant reduction in triglycerides, total cholesterol, LDL, VLDL, Atherosclerosis index and increase HDL. Atherogenesis results from high plasma triglycerides, total cholesterol, LDL, VLDL and low HDL which is one of the causes of cardiovascular diseases (Aggarwal et al., 2016a). A rise in HDL is one of the most important criteria for the anti-atherogenic agent as the high level of HDL inhibits LDL oxidation and protects the endothelial cell from the cytotoxic effect of LDL oxidation (Cedo et al., 2015). The decrease LDL oxidation reduces oxidative stress and therefore undermines atherogenesis. The fact that group 9 had the lowest atherosclerotic index might be due to the protective potential of the biscuit against inducing cardiovascular diseases (Aggarwal et al., 2016a).

The results (Table 5) of the serum creatinine and urea show that diabetic rats not taking optimum biscuit (group 4) had significantly highest urea and creatinine levels when compared to the other groups. Also, diabetic rats upon consumption of optimum biscuit with Metformin (group 9) intake reduced serum creatinine and urea. The increased level of urea and creatinine in the serum of diabetic rats indicates the reduction in the functioning of the kidney to filter waste products from blood and excrete them in urine (Sodipo et al., 2020). The reducing creatinine and urea levels in diabetic rats taking biscuits and Metformin is an indication that in addition to the known positive effect of Metformin, the biscuit can protect the kidney from damage. According to Abovwe et al. (2010), the reduced creatinine level in treated diabetic rats indicates a higher level of hemoglobin and thus collating with the anti-anemic effect of the biscuit. Similar results were obtained by Erukainure et al. (2013b) where they found that fibre-enrich cake had a therapeutic effect against biochemical changes associated with diabetes mellitus.

It can also be noted from Table 5 that, there was a significant difference between the ASAT, ALAT, ALP, BIL and total protein level of non-diabetic rats (group 1,2 and 3), diabetic rats fed on standard diets only (group 4) and diabetic rat fed on standard diet substituted with an optimum biscuit with/without Metformin (group 5,6,7,8 and 9). Nonetheless, apart from the total protein, the untreated diabetic group had the highest values for all the parameters. Also, group 9 had the lowest significant values for these parameters when compared to other diabetic group counterparts. Induction of diabetes results in the onset of oxidative stress in many tissues among which we have the liver. This leads to the peroxidation of membrane lipids which alter hepatic lipid profile which in turn lead to cellular damages and membrane rupture, causing the release of ASAT, BIL, ALT, and ALP into the bloodstream (Erukainure et al., 2015). Concerning total protein (TP), the decline in experimentally induced diabetic rats may be due to microproteinuria, which is a

Table 6. Effect of consumption of optimum biscuit on hematological parameters of diabetic and non-diabetic rats fed for 28 days.

Groups	RBC (10^6)/µL	HGB (g/dl)	PCV (%)	MCH (pg)	MCHC (g/dl)	MCV (fL)	PLT (10^3)/µL	LYM (%)	GRAN (%)	WBC (10^3)/µL
1	5.91 ± 0.22^{b}	17.35 ± 0.24^{b}	56.50 ± 1.13^{c}	29.41 ± 1.13^{d}	30.71 ± 0.54^{c}	95.73 ± 2.04^{d}	299.50 ± 0.58^{e}	39.60 ± 0.41^{c}	56.52 ± 1.77^{c}	6.18 ± 0.24^{ab}
2	5.96 ± 0.05^{bc}	17.13 ± 0.46^{b}	55.33 ± 0.70^{b}	28.76 ± 0.99^{cd}	30.96 ± 0.82^{c}	92.91 ± 1.47^{cd}	286.25 ± 2.06^{c}	38.50 ± 0.90^{b}	54.75 ± 0.47^{b}	7.05 ± 0.21^{c}
3	6.49 ± 0.20^{e}	17.04 ± 0.43^{b}	56.18 ± 0.55^{bc}	26.28 ± 0.89^{b}	30.34 ± 0.91^{bc}	86.66 ± 3.13^{ab}	290.60 ± 2.07^{cd}	39.14 ± 0.61^{bc}	54.96 ± 0.57^{b}	6.60 ± 0.38^{bc}
4	1.89 ± 0.17^{a}	4.43 ± 0.05^{a}	15.30 ± 0.29^{a}	23.60 ± 2.68^{a}	28.93 ± 0.41^{a}	81.61 ± 9.50^{a}	98.00 ± 2.16^{a}	18.22 ± 0.48^{a}	39.95 ± 0.76^{a}	5.70 ± 0.08^{a}
5	6.16 ± 0.15^{cd}	16.90 ± 0.70^{b}	55.47 ± 0.74^{bc}	27.44 ± 1.08^{bc}	30.46 ± 0.95^{bc}	90.08 ± 2.23^{bcd}	275.25 ± 2.87^{b}	39.15 ± 0.48^{bc}	60.87 ± 0.90^{d}	8.23 ± 0.81^{d}
6	6.20 ± 0.16^{d}	17.27 ± 0.61^{b}	55.60 ± 0.67^{bc}	27.85 ± 0.66^{bcd}	31.06 ± 0.87^{c}	89.71 ± 2.17^{bc}	288.50 ± 0.58^{c}	39.07 ± 0.81^{bc}	61.50 ± 0.35^{de}	8.30 ± 0.47^{d}
7	6.18 ± 0.17^{cd}	17.05 ± 0.58^{b}	55.85 ± 0.80^{bc}	27.61 ± 0.58^{bc}	30.53 ± 1.04^{bc}	90.48 ± 2.59^{bcd}	293.25 ± 5.56^{d}	38.40 ± 0.92^{b}	61.48 ± 0.55^{de}	8.43 ± 0.44^{d}
8	6.16 ± 0.13^{cd}	16.84 ± 0.62^{b}	55.50 ± 0.64^{bc}	27.32 ± 0.97^{bc}	30.34 ± 0.87^{bc}	90.06 ± 1.93^{bcd}	286.00 ± 3.08^{c}	38.78 ± 0.51^{bc}	62.16 ± 0.47^{ef}	8.84 ± 0.25^{d}
9	6.20 ± 0.07^{d}	16.64 ± 0.23^{b}	56.46 ± 0.39^{c}	26.85 ± 0.40^{b}	29.47 ± 0.41^{ab}	91.11 ± 1.60^{bcd}	294.20 ± 4.38^{d}	38.84 ± 0.61^{bc}	62.92 ± 0.68^{f}	8.38 ± 0.52^{d}

Values (mean ± standard deviation, n = 4) with different superscript letters in the same column are significantly different at $p<0.05$. group 1: Non diabetic rats fed with 100% standard diet only, group 2: Non diabetic rats fed with 50% standard diet and 50% biscuit, group 3: Non diabetic rats fed with 25% standard diet and 75 % biscuit, group 4: Diabetic rats fed with 100% standard diet only, group 5: Diabetic rats fed with 50% standard diet and 50% biscuit group 6: Diabetic rats fed with 25% standard diet and 75% biscuit, group 7: Diabetic rats fed with 100% standard diet and Metformin at 40 mg/kg, group 8: Diabetic rats fed with 50% standard diet, and 50% biscuit and Metformin at 40 mg/kg, group 9: Diabetic rats fed with 25% standard diet and 75% biscuit and Metformin at 40 mg/kg.

significant systematic indicator of diabetic nephropathy, or to augmented protein catabolism (Latha and Daisy, 2011). Thus the evaluation of these parameters would give diagnosis on the biological and xenobiotic-induced systemic toxicity (Pitocco et al., 2013). It has to be noted that, the values of ASAT, BIL, ALT, ALP and TP obtained in the non-diabetic groups (1, 2 and 3) and diabetic groups (5, 6, 7, 8 and 9) are in the normal range of values proposed by Giannini et al. (1999) and Diana (2007). These observations imply that the formulated biscuit is suitable for consumption and its consumption does not result in damage of the liver cell. As shown by Ajiboye et al. (2018) that Musa species diets could reduce the activities of the liver enzymes and thus probably prevent liver damage. Thus the biscuit may help to protect the induction of hepatoxicity in diabetes as it is made up of '*banane cochon*' which is a Musa species.

3.2.5. *Effect of consumption of optimum biscuit on hematological parameters of diabetic and non-diabetic rats*

The result of the hematological parameters of diabetic and non-diabetic rats are presented in Table 6. We can infer from this table that, there is a general significant difference in the number of WBC, % lymphocytes, RBC, granulocytes, hemoglobin, MCH, MCV, MCHC and PLT of diabetic and non-diabetic rats. Also, the WBCs, HCT, RBC, PLT, lymphocytes, granulocytes and Hb values of untreated diabetic rats were significantly lower when compared to the non-diabetic and treated diabetic rats with optimum biscuit and/or Metformin. HCT is the erythrocyte volume fraction of the percentage of red blood cells. RBC cells are involved in the transport of oxygen and absorbed nutrients (Sodipo et al., 2020). Thus a high HCT value shows better transportation, results in primary and secondary polythaemia and a low level indicates anaemia (Sodipo et al., 2020). WBC and its differentials on the other hand help in defense by phagocytosis, transport and distribution of immune antibodies leading to immunity. Thus groups with low values are exposed to a high risk of infections (Soetan et al., 2013). It has to be noted that, the values of the WBC, lymphocytes, RBC, granulocytes, hemoglobin, MCH, MCV, MCHC and PLT obtained in this study are in the range of values proposed by Fagbohuna et al. (2020) on streptozotocin-induced rats treated kigelia Africana fruit. The fact that diabetic groups fed on standard diet and substituted with optimum biscuit with/without Metformin were able to maintain their hematocrit level may be due to the micronutrient (Zn and Fe) which can boost the immune system Erukainure et al. (2013b).

3.2.6. *Histological examination of the pancreas*

Figure 1 shows the pancreas of diabetic and non-diabetic rats fed for 28 days. This figure reveals that there was a decrease in the population of beta-cells in non-treated diabetic rats (group 4) when compared to other groups. It has to be noted that, the non-diabetic groups (groups 1, 2 and 3) show normal cyto-structural components of the pancreas with an adequate population of pancreatic beta-cells. Also, the mild number of pancreatic beta-cells were observed in groups 5 and 6. Nonetheless, diabetic groups (7, 8 and 9) have similar pancreatic histology as the normal control. The pancreatic islet beta-cells are responsible for the production of insulin which in turn controls the level of serum glucose in the body (Akinlolu et al., 2015). Streptozotocin treatment leads to almost the destruction of the islet beta cells of non-treated diabetic rats. These results are in agreement with the study of Chaudhary and Mudgal (2020) that showed that a single dose of 55 mg/kg streptozotocin is capable of inducing the destruction of pancreatic beta cells of rats. Also, the presence of a normal pancreas with an adequate population of islet cells (non-diabetic groups) is due to the lack of induction with streptozotocin in this group. Furthermore, the examination showed pancreatic hyperplasia of groups 7, 8 and 9 with adequate islet beta-cells, atrophied and reduced hyperplasia of pancreatic islet beta-cells of groups 5 and 6. This finding implied that treatment of streptozotocin-diabetic rats with optimum biscuit with/without Metformin was able to facilitate the slowdown of the destruction (protection) of islet beta cells which corroborate

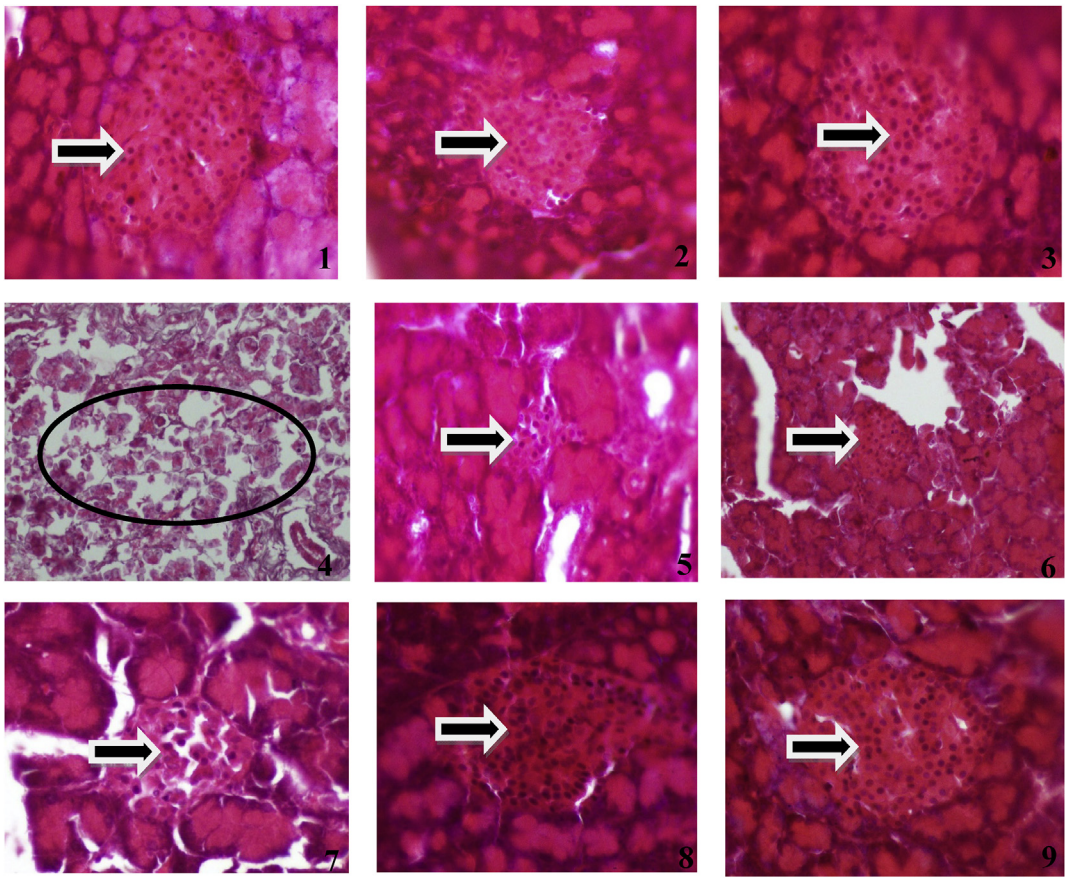

Figure 1. Pancreatic Histology of experimental rats after 4 weeks (haematoxylin and eosin stained 40X), 1 = group 1: Non diabetic rats fed with 100% standard diet only, 2 = group 2: Non diabetic rats fed with 50% standard diet and 50% biscuit, 3 = group 3: Non diabetic rats fed with 25% standard diet and 75 % biscuit, 4 = group 4: Diabetic rats fed with 100% standard diet only, 5 = group 5: Diabetic rats fed with 50% standard diet and 50% biscuit 6 = group 6: Diabetic rats fed with 25% standard diet and 75% biscuit, 7 = group 7: Diabetic rats fed with 100% standard diet and Metformin at 40 mg/kg, 8 = group 8: Diabetic rats fed with 50% standard diet, and 50% biscuit and Metformin at 40 mg/kg, 9 = group 9: Diabetic rats fed with 25% standard diet and 75% biscuit and Metformin at 40 mg/kg, arrows showing pancreatic beta cells, circle showing fatty cells with little or no pancreatic beta cells.

Practical application

Diabetic patients always find it difficult to have foods that contain health-benefiting ingredient(s) that also provide them with the necessary energy without varying their blood glucose level. In this study we assessed the effects of consumption of an optimum biscuit from composite flour of *Musa sapientum* (‘*banana cochon*’) and *Vigna unguiculata* (cowpea) flour, on the pancreatic histology, biochemical and hematological parameters of streptozotocin-induced diabetic *Wistar* rats. Our findings suggest that this biscuit could improve the health status (such as providing immunity as it contains nutrients required for the body functioning) of diabetic rats. Hence, the conditions required to obtain this biscuit can be exploited by food industries to provide biscuits to diabetic patients that could improve their health status and not impact their blood glucose level.

with the reduced blood glucose in these groups. This is in agreement with previously reported pancreatic histoprotection of different parts of *Musa sapientum* species and legume (cowpea), as they contain phytochemicals compounds that reduce oxidative stress (Akinlolu et al., 2015). The observed results in this study are in the same token as that obtained by Aggarwal et al. (2016a) where whole grains and fibre were able to protect the destruction of beta-cells mass.

4. Conclusion

This study portrays that optimum biscuit from *Musa sapientum* (*banane cochon*) and *Vigna unguiculata* (cowpea) composite flour is a low glycaemic index product rich in various nutrients. Generally, the biscuit had no harmful effect on vital organs and could safely be used either alone or in combination with metformin to improve the health of diabetic patients.

Declarations

Author contribution statement

Bilkissou Njapndounke: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Marc Bertrand Dandji Saah, Marius Edith Foko Kouam, Gires Teboukeu Boungo: Analyzed and interpreted the data; Wrote the paper.

Francois Zambou Ngoufack: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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