

Study of the Nutritional and Hypoglycemic Effects of *Soumbara* (*Parkia biglobosa*) and *Moringa* (*Moringa oleifera*) Leaf Formulations on Mouse (*Mus musculus*)

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Received August 30, 2023; Revised September 30, 2023; Accepted October 07, 2023

Abstract In Africa, and particularly in Ivory Coast, metabolic diseases represent a public health problem. Face at this problem the prevention seems to be a suitable solution. The objective of this work was to evaluate the anti-diabetic properties and nutritional effects of different formulations made from *soumbara* and *Moringa oleifera* leaves through an animal experiment. For this purpose, ten batches of five mouse were constituted. The animals of nine batches were made diabetic by alloxane. Mouse in lot 3 were given daily doses of glibenclamide at 5 mg/Kg bw and lots 4, 5, 6, 7, 8, 9 and 10 were given the aqueous extract of the formulations at 5000 mg/Kg bw for 21 days. The blood glucose levels of the mouse were measured. At the end of the experiment, the mice were fasted and anaesthetized with chloral and then sacrificed. Their blood was collected in dry tubes to determine their biochemical parameters. Thus, the results showed that the mouse which received by gavage the aqueous extracts of the diets particularly the formulation S20M80 and S30M70, proved to be more effective in the treatment of hyperglycemia. The observed results showed overall, an increase in body weight (2.33 to 14.33 g), serum iron level (78.25 to 96.41 µg /mL), but also a decrease in blood glucose level (1.70 to 1.16 g/l). Based on these results, the S20M80 formulation seemed to be suitable to help prevent type 2 diabetes and reduce micronutrient deficiencies.

Keywords: *Moringa* leaves, *soumbara*, food formulation, biochemical parameters

Cite This Article: Karnon COULIBALY, Fatoumata CAMARA, Ekoua Regina KRABI, Wahauwouele Hermann COULIBALY, Brahma KANDE and Koffi Cyrille TAN, "Study of the Nutritional and Hypoglycemic Effects of *Soumbara* (*Parkia biglobosa*) and *Moringa* (*Moringa oleifera*) Leaf Formulations on Mouse (*Mus musculus*).", *American Journal of Food and Nutrition*, vol. 11, no. 3 (2023): 89-99. doi: 10.12691/ajfn-11-3-4.

1. Introduction

Herbal medicine is enjoying an exceptional resurgence, especially in the treatment of chronic diseases such as diabetes [1]. Diabetes is a metabolic disease that High Health Authority become a public health problem [2]. Globally, the International Diabetes Federation estimates that 537 million people will have diabetes in 2021, compared with 150 million in 2000 and 366 million in 2010 [3]. This figure will rise to 643 million in 2030 and 743 million in 2045. In addition, 6.7 million people with diabetes died in 2021, an increase of 2.5 million in 2 years [4]. Thus, diabetes could be the 7th leading cause of death worldwide by 2030 [5]. Epidemiological forecasts estimate that the prevalence of diabetes will have

increased by 98 % by 2030 in sub-Saharan Africa [6]. In Côte d'Ivoire, diabetes represents a major public health problem due to its high prevalence. This prevalence was estimated at 4.9 % in 2014 [2] and 6.2 % in 2021, either 700,000 people in the population [7]. The treatment of diabetes long remained restricted to dietary changes, insulin injections and oral anti-diabetic agents [8]. The excessive cost of these anti-diabetic medicines and the inadequacy of medical infrastructures, combined with the lack of healthcare personnel in Africa are leading populations to turn to traditional medicine. Medicinal plants represent a medical potential that is accessible, available and inexpensive [9]. These plants are an inexhaustible resource, providing the majority of active ingredients in pharmaceutical products. However, many of the medicinal plants used lack scientific data on their efficacy and safety [10]. Indeed, for these plants to be

used rationally, work needs to be done to determine the possible positive effects induced by their use. With this in mind, we set out to study the effects of aqueous extracts of formulations based on *néré soumbara* (*Parkia biglosa*) and *Moringa oleifera* leaves. These two plants in the Ivorian pharmacopoeia are used in the treatment of several metabolic pathologies, including diabetes. Studies on the use of *Moringa oleifera* leaf extracts have confirmed hypoglycemic properties in patients with type 2 diabetes [11,12]. Results from *in vitro* studies suggest that regular intake of these leaves in the diet may protect diabetic patients against oxidative damage [13]. *Moringa oleifera* possesses anti-inflammatory and analgesic properties [14]. As for *néré soumbara* (*Parkia biglosa*), a study on the possible anti-diabetic and antihyperlipidemic effect of the extract of fermented *Parkia biglobosa* grains on alloxan-induced diabetic rats was carried out by Odetola et al. [15]. These authors concluded that the aqueous and methanolic extracts of fermented *Parkia biglobosa* seeds exerted a hypoglycemic effect, antidiabetic properties and probably anti-arteriogenic properties. Furthermore, the study by Modupe et al. [16] on the effect of *Parkia biglobosa* extract on open skin wound healing in dexamet. Induced hyperglycemia and histological evaluation in rats showed that the three different doses (25, 50 and 100 mg/kg body weight) of *P. biglobosa* extract decreased serum glucose concentration in dexamet. The aim of this work was to evaluate the nutritional and therapeutic effects of different formulations based on *néré soumbara* and *Moringa oleifera* leaves in the treatment of diabetes mellitus, by studying the effects of their aqueous extracts on mice made diabetic by alloxane and assessing their serum parameters.

2. Material and Methods

2.1. Plant Material

The plant material used consists of formulations made from *soumbara* (*Parkia biglobosa*) and *Moringa oleifera* leaves. The formulations were prepared according to the method described by Karnon et al [17].

2.2. Animal Material

Mouse of the species *Mus musculus*, 12 weeks old and weighing on average 20 to 25 grams were used. These mice were fed mainly with pellets manufactured by IVOGRAIN Abidjan (Ivory Coast) and lived at an average room temperature of 28 °C, in an atmosphere containing 65 % humidity, the photoperiod was 12 hours/24 hours.

2.3. Chemical Products

Chloroform (merck, Germany) was used to anesthetize the animals. Alcohol 90° was used for sterilization. Alloxane monohydrate 98 % Sigma (France) was used to induce diabetes. Glibenclamide (Glidiabet ferrer international SA, Spain) was used as a reference hypoglycemic medicine.

2.4. Experimental Diabetes Induction

The method used is that described by Nagapp et al [18] with slight modifications.

Normal glycemic mouse were fasted for 18 hours and made diabetic by intraperitoneal injection of alloxane (dissolved in 9 % NaCl, at a dose of 200 mg/kg). After injection of alloxane the mouse had free access to food and water, and they received 5 % anhydrous glucose solution by gavage to prevent hypoglycemic shock. After 72 hours, fasting blood glucose levels were determined and only mouse with blood glucose levels above 180 mg/dL were selected for further experimentation.

For each formulation with the powder of the fermented fruit 'soumbara' of *Parkia biglobosa* and the powder of the leaves of *Moringa oleifera*, an aqueous solution concentration 50 g/L was prepared. For this, 50 grams of powder of each formulation were boiled for 15 minutes, in 1000 mL of distilled water. The resulting preparations were used to treat animals by intragastric gavage.

2.5. Evaluation of the Effects of the Aqueous Extract of the Formulations Made with *Soumbara* and *Moringa Oleifera* Leaves on the Glycemia in Mouse made Diabetic by Alloxane

Fifty (50) mouse divided into ten (10) batches of five (05) mouse of relatively homogeneous weight were used.

Lot 1 consisted of normal, non-diabetic mice, the normoglycemic controls (NGC), receiving one (01) mL of distilled water for the duration of the experiment.

Lot 2 consisted of diabetic mice, which were the diabetic controls (DC), treated with one (01) mL of distilled water throughout the experiment.

Lot 3 consisted of diabetic control mouse treated with a daily dose of 5 mg/kg body weight of Glibenclamide (TG), the reference hypoglycemic substance.

Lot 4 included diabetic mouse treated with aqueous extract of *soumbara* (S100).

Lot 5 included diabetic mouse treated with *Moringa oleifera* aqueous leaf extract (M100).

Lot 6 included diabetic mouse that received the aqueous extract of the formulation made from 80 % *soumbara* and 20 % *Moringa oleifera* leaves (S80M20).

Lot 7 included diabetic mouse that received the aqueous extract of the formulation based on 20 % *soumbara* and 80 % *Moringa oleifera* leaves (S20M80).

Lot 8 included diabetic mouse that received the aqueous extract of the formulation based on 70 % *soumbara* and 30 % *Moringa oleifera* leaves (S70M30).

Lot 9 included diabetic mouse that received the aqueous extract of the formulation based on 30 % *soumbara* and 70 % *Moringa oleifera* leaves (S30M70).

Lot 10 included diabetic mouse that received the aqueous extract of the formulation based on 50 % *soumbara* and 50 % *Moringa oleifera* leaves (S50M50).

These different batches of mouse were gavaged daily during the experiment with one (1) mL of 5000 mg/kg body weight of each corresponding aqueous extract. Blood glucose levels were measured for each batch on days 1, 3, 12 and 21 of treatment using an ACON Diabetes "On Call Extra" strip glucose meter. Results are expressed in mg/dL of blood.

2.6. Collection of Blood Samples and Determination of Biochemical Parameters and Serum Ions

At the end of the experiments, the animals were fasted for 16 hours before blood sampling [19]. The following day, they were anesthetized with 10 % chloral at a dose of 3 mL/Kg, then sacrificed. Blood samples were taken in the morning between 8 and 10 am. Approximately 2 to 3 mL of blood were collected in dry tubes without anticoagulant. Blood samples were centrifuged at 3,000 rpm/10 min, and the collected serum was stored at -20 °C for biochemical and electrolyte assays.

Biochemical assays in serum were performed using an automatic multiparameter analyzer (Hitachi 902, Germany). Assay methods differed according to the biochemical parameter sought. Assays covered serum metabolites such as urea, uric acid, glucose, creatinine, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, bilirubins and enzymes (ALAT, ASAT), glutathione peroxidase, superoxide dismutase, antioxidant status, and p^{5+} , Na^{+} , K^{+} , Fe^{3+} , and Ca^{2+} ions.

2.7. Statistical Analysis

An analysis of variance was performed with the XLSTAT software (Version 2016; Adinosoft Inc.) and differences between mean values were determined by Tukey's test ($P < 0.05$). In order to regroup formulations which have the same characteristics, dendrogram was done using XLSTAT software.

3. Results and Discussion

3.1. Effects of Consumption of Formulations made from *Soumbara* and *Moringa* Leaves on Hyperglycemia in Mice.

The effect of the formulations on induced hyperglycemia in mice is shown in Figure 1. Analysis of the results showed no significant variation ($p > 0.05$) in mean blood glucose levels on the first day of the experiment. However, on subsequent days, blood glucose levels increased significantly ($p < 0.05$) compared with the normoglycemic control (TNG) batch.

The average blood glucose level of the mouse ranged from 0.55 ± 0.04 g/L to 1.04 ± 0.45 g/L on the first day of the experiment. After induction of hyperglycemia in the mice, mean blood glucose levels ranged from 2.16 ± 0.04 g/L to 2.01 ± 0.08 g/L, with the exception of the normoglycemic control batch (0.90 ± 0.06 g/L). After nine (9) days of treatment, a decrease in blood glucose levels was observed in all batches of mouse compared with the untreated diabetic control (DC) batch. The mean blood glucose level of the untreated diabetic control was higher (2.89 ± 0.06 g/L), while the mean blood glucose level of the normoglycemic control was lower (0.93 ± 0.04 g/L), followed by the control treated with glibenclamide (TG) (1.31 ± 0.02 g/L), then the batches of mouse subjected to formulations S20M80 (1.41 ± 0.05 g/L) and S30M70 (1.52 ± 0.04 g/L). Mouse fed formulations S70M30 (1.74

± 0.03 g/L), S80M20 (1.78 ± 0.60 g/L) and S50M50 (1.69 ± 0.14 g/L) had higher blood glucose levels than the previous formulations, but no significant difference was observed between them ($p > 0.05$). At the end of the experiment, the mean blood glucose level of the untreated diabetic control was still high (2.89 ± 0.02 g/L) compared with the other batches. The normoglycemic control had a lower mean blood glucose level (0.88 ± 0.10 g/L), followed by the glibenclamide-treated batch (1.08 ± 0.08 g/L) and those treated with formulations S20M80 (1.16 ± 0.05 g/L) and S30M70 (1.21 ± 0.35 g/L). For mouse fed formulations S70M30 (1.53 ± 0.45 g/L), S80M20 (1.62 ± 0.03 g/L) and S50M50 (1.44 ± 0.20 g/L), mean blood glucose levels changed in virtually the same way. This reduction in blood glucose levels suggests that *soumbara* and *Moringa* leaves, in particular formulations S20M80 and S30M70, have a real antihyperglycemic effect. Such an effect. High Health Authority been demonstrated by other authors when treating diabetic rats with extracts of plants such as *Musanga cecropioides* and *Berberis aristata* [20,21] and alloxanized rats at different doses. Indeed, the reduction in blood glucose levels could be due to the insulin secreted, which would have stimulated the expression of genes involved in glucose utilization (glucokinase L-pyruvate kinase, biogenic enzymes) and inhibited the expression of genes involved in glucose production (phosphoenolpyruvate, carboxykinase) [22]. This reduction in glycemia could be beneficial for diabetics.

Moreover, this stimulation would only be possible thanks to the presence of phytochemical compounds in formulations such as flavonoids, tannins and polyphenols. In addition, phenolic compounds could have a protective effect on hyperglycemia [23]. Authors such as Villarruel-López et al. [24] have demonstrated a hypoglycemic effect of *Moringa oleifera* dried leaf powder. These authors administered 50 mg/day of leaf powder from this plant species for eight (8) weeks to alloxane-induced diabetic rats. This administration led to a reduction in blood glucose levels in the second week, which tended to be maintained in subsequent weeks. The antihyperglycemic activity observed could be attributed to flavonoids and tannins. Authors have demonstrated that flavonoid extracts from plants such as *Eugenia jambolana*, *Cassia auriculata* L and *Teucrium polium* stimulate and regenerate pancreatic β -cells involved in blood sugar regulation [25-27]. Clearly, these formulations contain high levels of polyphenolic compounds with capillary protection and antioxidant properties. Formulations based on *soumbara* and *Moringa* leaves, in this case formulation S20M80, could be beneficial to people with diabetes.

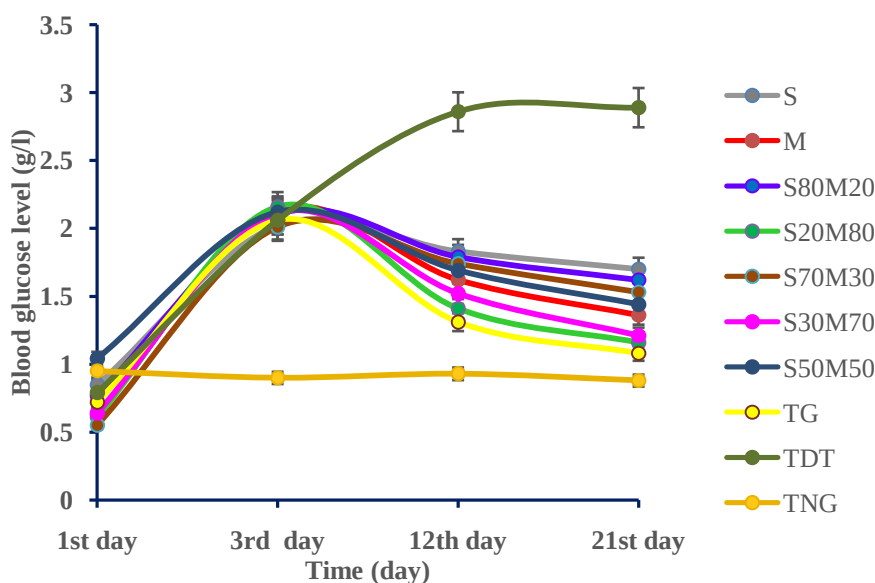
3.2. Effects on Body Weight of Mouse Fed Formulations Based on *Soumbara* and *Moringa* Leaves.

Changes in body weight of mouse fed the formulated diets are shown in Figure 2. The initial weights of mouse fed the diets ranged from 19.33 ± 1 g to 20.33 ± 2.64 g. However, after 21 days of experimentation, the weight of the mouse varied significantly ($p < 0.05$). This weight gain ranged from 8 ± 0 g to 14.33 ± 0.42 g in the different batches of mouse compared with the untreated diabetic

control batch. Thus, a significant weight gain was observed in the normoglycemic control (14.33 ± 0.42 g) and the batch given *soumbara* (8 ± 0 g). Weight loss was then observed in the untreated diabetic control (-2.66 ± 0.37 g).

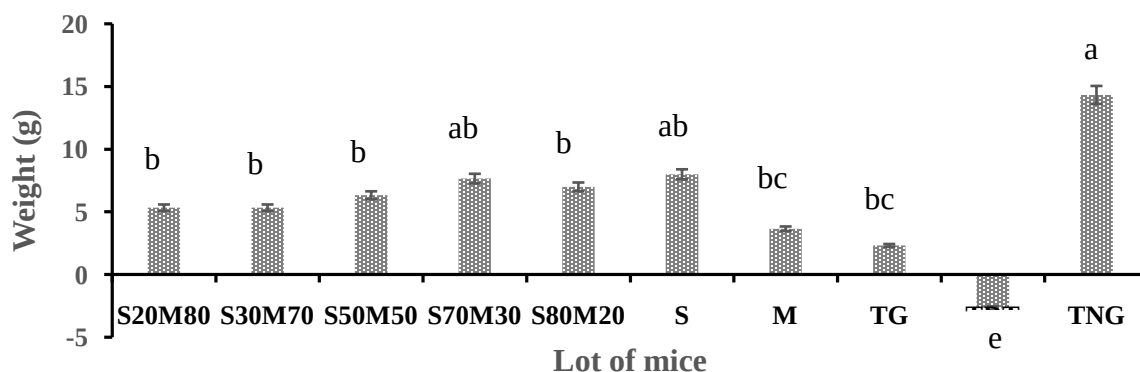
This difference could be explained by lipolysis and disruption of insulin secretion due to alloxane-induced diabetes. However, body weight gain was observed in diabetic mouse fed the formulated diet compared with those treated with glibenclamide. This weight gain is thought to be due to the high protein content of the *soumbara* and *Moringa oleifera* leaf feeds. Protein is an essential nutrient for harmonious organ growth [28]. Ekpo et al. [29] also observed a progressive increase in the weight of rats after administration of aqueous extract of

Ipomea batatas. Osman et al. [30] noted an increase in body weight in rats after administration of aqueous *Moringa oleifera* leaf extract. For these authors, the increase in body weight was due to the fact that *Moringa oleifera* leaves are rich in amino acids, vitamins and minerals, particularly iron. But it could also be attributed to the rats' captivity, where energy expenditure is minimal. This weight gain is in line with the finding of Ekundina et al. [31], who observed an increase in body weight in rats after administration of ethanolic extract of *Moringa oleifera* leaves. Consequently, consumption of the formulated foods could be recommended to people suffering from weight loss.



(TNG: Normoglycemic controls; TDT: Untreated diabetic controls; TG: Glibenclamide-treated diabetic controls; S: *Soumbara* -treated diabetic mice; M: *Moringa oleifera* leaf-treated diabetic mice; S80M20: Diabetic mouse treated with the formulation made from 80 % *soumbara* and 20 % *Moringa oleifera* leaves; S20M80: Diabetic mouse treated with the formulation made with 20 % *soumbara* and 80 % *Moringa oleifera* leaves; S70M30: Diabetic mouse treated with the formulation made with 70 % *soumbara* and 30 % *Moringa oleifera* leaves; S30M70: Diabetic mouse treated with the formulation made with 30 % *soumbara* and 70 % *Moringa oleifera* leaves; S50M50: Diabetic mouse treated with the formulation made with 50 % *soumbara* and 50 % *Moringa oleifera* leaves).

Figure 1. Effects of consumption of formulations made from *soumbara* and *Moringa* leaves on hyperglycemia in mice



(TNG: Normoglycemic controls; TDT: Untreated diabetic controls; TG: Glibenclamide-treated diabetic controls; S: *Soumbara* -treated diabetic mice; M: *Moringa oleifera* leaf-treated diabetic mice; S80M20: Diabetic mouse treated with the formulation made from 80 % *soumbara* and 20 % *Moringa oleifera* leaves; S20M80: Diabetic mouse treated with the formulation made with 20 % *soumbara* and 80 % *Moringa oleifera* leaves; S70M30: Diabetic mouse treated with the formulation made with 70 % *soumbara* and 30 % *Moringa oleifera* leaves; S30M70: Diabetic mouse treated with the formulation made with 30 % *soumbara* and 70 % *Moringa oleifera* leaves; S50M50: Diabetic mouse treated with the formulation made with 50 % *soumbara* and 50 % *Moringa oleifera* leaves).

Figure 2. Effects on body weight of mouse fed formulations based on *soumbara* and *Moringa* leaves

3.3. Effects of *Soumbara* and *Moringa* Leaf Formulations on Changes in Live Weight of Mice

The variation in live weight of mouse fed formulated diets is shown in Table 1. The initial weights of mouse fed the diets ranged from 19.33 ± 1 g to 20.33 ± 2.64 g. However, after 5 days of experimentation, the weight of the mouse did not vary significantly. From day 11

onwards, a significant weight change was observed ($P < 0.005$). Non-diabetic mouse (TNG) grew by 0.36 ± 0.73 g and those treated with the S50M50 formulation by 0.27 ± 0.15 g. In contrast, negative growth (-0.39 ± 0.64 g) was recorded in mouse treated with glibenclamide. A significant difference ($P < 0.005$) was observed on day 21 of treatment. Thus, the batch of mouse treated with *P. biglobosa* recorded a growth of 0.38 ± 0.1 g.

Table 1. Variation in live weight of mouse over the course of the experiment.

Lot of mice	5 th day	11 st day	17 th day	21 st day
S80M20	0.73 ± 0.46^a	0.06 ± 0.57^c	0.25 ± 0.2^b	0.33 ± 0.52^b
S20M80	0.6 ± 0.08^a	0.06 ± 0.1^c	0.15 ± 0.73^{bc}	0.25 ± 0.08^b
S70M30	0.33 ± 0.57^a	0.15 ± 0.57^{bc}	0.27 ± 0.57^b	0.36 ± 0.57^{ab}
S30M70	0.6 ± 0.2^a	-0.12 ± 0.05^e	0.17 ± 0.35^{bc}	0.25 ± 0.30^b
S50M50	0.33 ± 0.15^a	0.27 ± 0.15^{ab}	0.29 ± 0.57^{ab}	0.30 ± 0.57^b
S	0.33 ± 0.15^a	0.24 ± 0.52^{ab}	0.33 ± 0.15^{ab}	0.38 ± 0.1^{ab}
M	0.4 ± 0.51^a	-0.03 ± 1^d	0	0.19 ± 0.73^{bc}
TG	0.46 ± 0.73^a	-0.39 ± 0.64^f	-0.03 ± 0.50^d	0.11 ± 0.11^{bc}
TDT	0.20 ± 0.35^a	-0.27 ± 0.64^{ef}	-0.23 ± 0.2^e	-0.11 ± 0.51^c
TNG	0.2^a	0.36 ± 0.73^a	0.52 ± 0.1^a	0.68 ± 0.57^a

TNG: Normoglycemic controls; TDT: Untreated diabetic controls; TG: Glibenclamide-treated diabetic controls; S: *Soumbara* -treated diabetic mice; M: *Moringa oleifera* leaf-treated diabetic mice; S80M20: Diabetic mouse treated with the formulation made from 80 % *soumbara* and 20 % *Moringa oleifera* leaves; S20M80: Diabetic mouse treated with the formulation made with 20 % *soumbara* and 80 % *Moringa oleifera* leaves; S70M30: Diabetic mouse treated with the formulation made with 70 % *soumbara* and 30 % *Moringa oleifera* leaves; S30M70: Diabetic mouse treated with the formulation made with 30 % *soumbara* and 70 % *Moringa oleifera* leaves; S50M50: Diabetic mouse treated with the formulation made with 50 % *soumbara* and 50 % *Moringa oleifera* leaves

3.4. Effects of Diets on Mouse Serum Electrolytes

Table 2 shows the results of the effect of formulations on serum electrolytes in mice. Analysis of the results showed that there was no significant variation ($p > 0.05$) in serum phosphorus and sodium levels. However, potassium levels varied significantly ($p < 0.05$). Thus, the batch of mouse treated with *soumbara* (4.77 ± 0.54 mEq/L) recorded the highest value and the untreated diabetic control (2.85 ± 0.29 mEq/L) the lowest. The other batches were statistically identical.

With regard to serum iron, a significant increase ($p < 0.05$) was observed in all batches of mouse treated with the formulations. Mouse treated with *Moringa* leaves (96.41 ± 1.10 μ g /mL) recorded the highest iron levels, followed by those treated with formulations S20M80 (92.06 ± 6.7 μ g /mL) and S50M50 (89.47 ± 3.5 μ g /mL). The untreated diabetic control (TD) showed the lowest iron levels (63.17 ± 11.51 μ g /mL).

A significant increase in serum calcium levels was observed across formulations. The S20M80 formulation (142.92 ± 20.41 mg/L) had the highest value, while the normoglycemic control had the lowest (59.48 ± 4.59 mg/L).

The significant increase ($p < 0.05$) in serum iron levels in the blood of mouse fed the formulated diets compared

with the various controls indicates that these diets contain substances capable of promoting iron intake. Serum iron is iron that is not bound by red blood cells, but is circulating in blood serum (plasma) [32]. In addition, iron is a constituent of heme and is associated with globin molecules to form hemoglobin in the bone marrow [33]. In other words, red blood cell count and serum iron are linked. According to the French National Authority for Health [32], an increase in serum iron levels in the blood would also reflect an increase in hemoglobin in red blood cells. Consumption of foods formulated from *soumbara* and *Moringa oleifera* leaves could be beneficial against iron-deficiency anemia.

On the other hand, serum phosphorus and potassium levels remain below the norm indicated by Harkness et al. [34] and Keeble, [35] and serum calcium levels are above the norm. However, plasma sodium levels were within the standard.

3.5. Effects of formulations on Mouse Serum Lipid Parameters

Serum lipid parameters in mouse are shown in Table 3. Cholesterol levels ranged from 1.10 ± 0.32 g/L to 1.63 ± 0.09 g/L. Triglyceride levels ranged from 1.32 ± 0.01 g/L for *soumbara* to 1.94 ± 0.23 g/L for the S50M50 formulation. HDL levels ranged from 0.27 ± 0.02 g/L for

the S30M70 formulation to 0.43 ± 0.06 g/L for the normoglyceride control.

No significant variation ($p > 0.05$) was observed in lipid parameters.

Analysis of the lipid profile showed that the formulated diets did not significantly impact triglycerides, total cholesterol and HDL cholesterol in mouse compared with the untreated diabetic control, the normoglycemic control and the glibenclamide control. Lipid balance is of crucial

importance in the treatment of cardiovascular disease and the control of diabetic patients [36]. Several studies have reported that cardiovascular complications associated with diabetes are due to disturbances in lipid metabolism [37]. Lowering lipid levels during diabetes therefore reduces the risk of cardiovascular complications [38]. *Soumbara* and *Moringa* leaf formulations did not significantly increase the lipid profile of mice.

Table 2. Effects of diets on serum electrolytes in mice

Lot of mouse	Phosphorus (mg/l)	Serum parameters			
		Potassium (mEq/l)	Sodium (mEq/l)	Iron (μ g/ml)	Calcium (mg/l)
S20M80	14.53 ± 2.44^a	3.94 ± 0.15^{ab}	114.95 ± 0.94^a	92.06 ± 6.70^{ab}	142.92 ± 20.41^a
S30M70	14.39 ± 3.81^a	3.42 ± 0.78^{ab}	113.10 ± 2.33^a	84.99 ± 9.21^{ab}	98.60 ± 3.58^b
S50M50	11.78 ± 0.53^a	3.64 ± 1.10^{ab}	117.27 ± 9.27^a	89.47 ± 3.55^{ab}	89.88 ± 11.61^{bc}
S70M30	15.89 ± 2.25^a	4.09 ± 0.19^{ab}	114.95 ± 12.96^a	78.25 ± 8.66^{ab}	88.85 ± 1.19^{bc}
S80M20	15.43 ± 4.90^a	3.60 ± 0.22^{ab}	133.29 ± 55.91^a	83.90 ± 1.10^{ab}	102.99 ± 17.90^b
S	16.49 ± 2.67^a	4.77 ± 0.54^a	133.35 ± 54.22^a	85.24 ± 8.99^{ab}	98.54 ± 2.69^b
M	15.88 ± 1.78^a	3.75 ± 0.14^{ab}	105.55 ± 7.59^a	96.41 ± 1.10^a	105.11 ± 7.82^b
TG	14.85 ± 1.15^a	3.39 ± 0.23^{ab}	102.60 ± 5.25^a	63.17 ± 11.51^b	55.08 ± 6.21^d
TDT	13.12 ± 3.74^a	2.85 ± 0.29^b	74.74 ± 17.78^a	61.63 ± 12.50^b	58.59 ± 3.20^{cd}
TNG	18.83 ± 0.83^a	3.57 ± 0.55^{ab}	99.14 ± 3.25^a	59.48 ± 4.59^b	77.25 ± 17.96^{bcd}
Norm	60.14-110.36	5.1-10.4	112-193	-	32.06 – 80.15

TNG: Normoglycemic controls; TDT: Untreated diabetic controls; TG: Glibenclamide-treated diabetic controls; S: *Soumbara* -treated diabetic mice; M: *Moringa oleifera* leaf-treated diabetic mice; S80M20: Diabetic mouse treated with the formulation made from 80 % *soumbara* and 20 % *Moringa oleifera* leaves; S20M80: Diabetic mouse treated with the formulation made with 20 % *soumbara* and 80 % *Moringa oleifera* leaves; S70M30: Diabetic mouse treated with the formulation made with 70 % *soumbara* and 30 % *Moringa oleifera* leaves; S30M70: Diabetic mouse treated with the formulation made with 30 % *soumbara* and 70 % *Moringa oleifera* leaves; S50M50: Diabetic mouse treated with the formulation made with 50 % *soumbara* and 50 % *Moringa oleifera* leaves

Table 3. Effect of formulations on serum lipid parameters in mice

Lot of mouse	Serum lipid parameters		
	Chol Total (g/l)	Triglyc (g/l)	HDL (g/l)
S20M80	1.30 ± 0.28^a	1.87 ± 0.30^a	0.32 ± 0.06^a
S30M70	1.10 ± 0.32^a	1.49 ± 0.70^a	0.27 ± 0.02^a
S50M50	1.25 ± 0.02^a	1.94 ± 0.23^a	0.31 ± 0^a
S70M30	1.55 ± 0.37^a	1.63 ± 0.34^a	0.38 ± 0.09^a
S80M20	1.50 ± 0.04^a	1.32 ± 0.01^a	0.37 ± 0.01^a
S	1.42 ± 0.54^a	1.76 ± 0.74^a	0.35 ± 0.13^a
M	1.41 ± 0.00^a	1.61 ± 0.20^a	0.35 ± 0^a
TG	1.49 ± 0.19^a	1.65 ± 0.22^a	0.37 ± 0.04^a
TDT	1.23 ± 0.09^a	1.95 ± 0.40^a	0.30 ± 0.02^a
TNG	1.63 ± 0.09^a	1.70 ± 0.16^a	0.43 ± 0.06^a

TNG: Normoglycemic controls; TDT: Untreated diabetic controls; TG: Glibenclamide-treated diabetic controls; S: *Soumbara* -treated diabetic mice; M: *Moringa oleifera* leaf-treated diabetic mice; S80M20: Diabetic mouse treated with the formulation made from 80 % *soumbara* and 20 % *Moringa oleifera* leaves; S20M80: Diabetic mouse treated with the formulation made with 20 % *soumbara* and 80 % *Moringa oleifera* leaves; S70M30: Diabetic mouse treated with the formulation made with 70 % *soumbara* and 30 % *Moringa oleifera* leaves; S30M70: Diabetic mouse treated with the formulation made with 30 % *soumbara* and 70 % *Moringa oleifera* leaves; S50M50: Diabetic mouse treated with the formulation made with 50 % *soumbara* and 50 % *Moringa oleifera* leaves

3.6. Effects of Formulations on Mouse Serum Metabolites

The results of the mean serum metabolite values of mouse fed the different diets are shown in Table 4. The mean urea values of the different batches of mouse were significantly different ($p < 0.05$). The untreated diabetic control showed a higher urea value (0.38 ± 0.03 g/L) within the range indicated by the standard (0.366-0.6 g/L). Subsequently, mouse treated with *soumbara* showed a low urea value (0.16 ± 0.02 g/L).

Serum creatinine levels changed significantly ($p < 0.05$) and these values were above the norm (3.051-3.164 g/L). The untreated diabetic control (TD) had the highest value (6.34 ± 1.02 g/L) compared with the batches of mouse treated with the formulated diets, and the normoglycemic control (TNG) had a low creatinine level (3.28 ± 0.14 g/L).

There was no significant difference in uric acid levels ($p > 0.05$). Mean uric acid values ranged from 37.14 ± 0.81 g/L for the untreated diabetic control to 25.99 ± 1.55 g/L for the control treated with glibenclamide (TG). Formulations S30M70 and S50M50 recorded 32.56 g/L and 32.18 ± 1.29 g/L respectively.

The increase in serum urea concentration in the untreated diabetic control would be explained by the degradation of protein compounds into amino acids and then into urea due to alloxane-induced proteolysis. Furthermore, serum urea levels are generally excessive in diabetics. These findings reflect activation of ureogenesis, a liver-specific function [39]. Urea is the final step in the catabolism of free amino acids. Serum levels of free amino acids are high in diabetics [40]. On the other hand, serum creatinine levels were close to those of controls given distilled water and glibenclamide, demonstrating that formulated foods do not affect kidney function.

The Aspartate Amino Transferase (ASAT) and Alanine Amino Transferase (ALAT) activity values of mouse fed the different diets were not significantly different ($p > 0.05$). The mean ASAT and ALAT values of mouse on the S50M50 diet (67.79 ± 24.86 IU/L and 56.71 ± 25.21 IU/L respectively) were higher and lower for mouse treated with glibenclamide (33.38 ± 21.47 IU/L and 23.51 ± 22.35 IU/L for ASAT and ALAT respectively). The mean ASAT values of mouse on the S50M50 diet (67.79 ± 24.86 IU/L) and the normoglycemic control (55.04 ± 0.12 IU/L) fell within the range indicated by the standard (54-269 IU/L). As for the mean ALAT value, the various batches of mouse were within the range set by the standard (27-77 IU/L), with the exception of mouse treated with *Moringa* leaves (26.05 ± 8.93 IU/L) and those treated with glibenclamide (23.51 ± 22.35 IU/L).

Liver enzymes such as ALAT and ASAT are assayed to determine the toxicity of formulated diets. A substantial increase in ASAT and ALAT values in the blood is a clear sign of cell lysis and loss of functional integrity of the hepatocyte membrane. Consumption of diets formulated with *soumbara* and *Moringa* leaves did not result in a significant change in ASAT and ALAT levels in mouse compared with the glibenclamide control and the normoglycemic control. The overall ASAT and ALAT values obtained were within the norm range (54-269 IU/L for ASAT and 27-77 IU/L for ALAT) reported by Harkness et al. [34] and Keeble, [35]. These results show

that diets formulated with *soumbara* and *Moringa* leaves did not induce inflammation and necrosis of liver hepatocyte cells.

With regard to total and conjugated bilirubin levels, mean values were only statistically different ($p > 0.05$) and within the range indicated by the standard (1.17-8.775 mg/L) for total bilirubin. Total bilirubin levels ranged from 3.15 ± 0.50 mg/L for mouse fed *soumbara* to 2.75 ± 0.99 mg/L for the S70M30 formulation. As for conjugated bilirubin, the mean value was 0.79 ± 0.17 mg/L for mouse on the S80M20 diet and 0.41 ± 0.27 mg/L for mouse on the S20M80 formulation.

If ALAT is the best marker of poor liver function, so is bilirubin [41]. Furthermore, consumption of the formulated diets does not result in any significant variation in total and conjugated bilirubin compared to controls, and mean total bilirubin values are within the norm range (1.17- 8.775 g/l) reported by Harkness et al. [34] and Keeble, [35]. In addition, total bilirubin levels were slightly reduced in mouse fed diets formulated with *soumbara* and *Moringa* leaves, compared with control batches. This reduction in total bilirubin could be due to the protective action of the phenolic compounds present in *soumbara* and *Moringa* leaves thanks to their antioxidant power.

3.7. Assessment of the Antioxidant System

The effect of the formulations on mouse superoxide dismutase is shown in Figure 3. Analysis of the results showed a significant difference ($p < 0.05$) in the superoxide dismutase levels of the different batches of mice. The normoglycemic control showed the highest mean value (188.26 ± 09 IU/L Hb) for erythrocyte superoxide dismutase, followed by mouse on formulas S20M80 (166.33 ± 1.20 IU/L Hb) and S30M70 (163.38 ± 1.08 IU/L Hb). However, the untreated diabetic control showed the lowest value (124.13 ± 1.52 IU/L Hb).

Results for glutathione peroxidase are shown in Figure 4. The glutathione peroxidase results show a significant difference ($p < 0.05$). Thus, the mean glutathione value was higher in the non-diabetic control (53.12 ± 0.53 IU/g Hb) and lower in the untreated diabetic control (26.24 ± 0.85 IU/g Hb). Glutathione was also more abundant in mouse on the S30M70 (41.21 ± 0.35 IU/g Hb) and S70M30 (40.57 ± 0.5 IU/g Hb) diets than in other batches of mice. Figure 5 shows the results for antioxidant status in mice. Analysis of the results showed a significant difference ($p < 0.05$) in the antioxidant status of the mice. In addition, mouse on the S20M80 diet had a higher antioxidant status (1.83 ± 0.33 mmol/L) than those treated with glibenclamide (1.72 ± 0.5 mmol/L). However, the antioxidant status of the non-diabetic control was higher (2.32 ± 0.60 mmol/L).

The antioxidant system balance showed a decrease in total antioxidant status, superoxide dismutase and glutathione peroxidase in diabetic mouse compared to the non-diabetic control, indicating the presence of a state of intense oxidative stress in diabetic mice. The decrease in superoxide dismutase activity can be explained by an increase in its glycation [42]. These results are not consistent with those of Sekeroglu et al. [43], who found an increase in erythrocyte superoxide dismutase in their

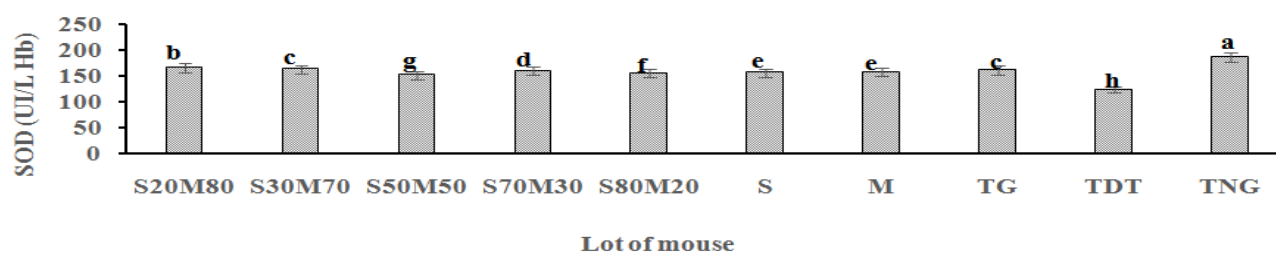
study. On the other hand, mouse fed the dietary formulas showed a higher level of superoxide dismutase than the untreated diabetic control, which would testify to the impact of the dietary formulas on the antioxidant system

of the mice. The decrease in glutathione peroxidase activity and total antioxidant status could be explained by an increase in glutathione peroxidase enzyme activity as a response to hyperglycemia.

Table 4. Effects of formulations on serum metabolites in mice.

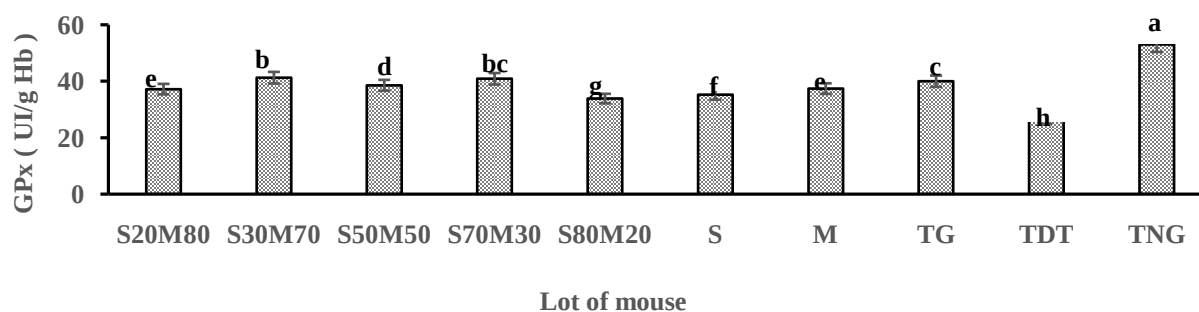
Lot of mouse	Serum parameters						
	Urea (g/l)	Creatinine (mg/l)	Uric acid (g/l)	T B (mg/l)	C B(mg/l)	ASAT (UI/l)	ALAT (UI/l)
S20M80	0.25 ± 0.09 ^{ab}	3.61 ± 0.22 ^b	31.05 ± 6.98 ^a	2.75 ± 0.44 ^a	0.41 ± 0.27 ^a	42.23 ± 13.31 ^a	31.75 ± 13.56 ^a
S30M70	0.22 ± 0.02 ^{ab}	3.55 ± 0.25 ^b	32.56 ± 0 ^a	2.83 ± 0.57 ^a	0.66 ± 0 ^a	39.13 ± 4.21 ^a	29.22 ± 7.12 ^a
S50M50	0.19 ± 0.03 ^b	3.55 ± 0.38 ^b	32.18 ± 1.29 ^a	3.06 ± 0.13 ^a	0.43 ± 0.47 ^a	67.79 ± 24.86 ^a	56.71 ± 25.21 ^a
S70M30	0.30 ± 0.10 ^{ab}	3.46 ± 0.30 ^b	29.60 ± 3.34 ^a	2.75 ± 0.99 ^a	0.47 ± 0.14 ^a	38.62 ± 16.04 ^a	28.22 ± 16.60 ^a
S80M20	0.18 ± 0.04 ^b	3.41 ± 0.05 ^b	33.53 ± 0.55 ^a	2.88 ± 0.59 ^a	0.79 ± 0.17 ^a	38.43 ± 2.15 ^a	25.54 ± 2.49 ^a
S	0.16 ± 0.02 ^b	3.45 ± 0.20 ^b	31.24 ± 1.91 ^a	2.99 ± 0.57 ^a	0.64 ± 0.20 ^a	45.74 ± 21.90 ^a	34.72 ± 21.96 ^a
M	0.21 ± 0.00 ^b	3.41 ± 0.01 ^b	32.10 ± 0.27 ^a	3.01 ± 0.65 ^a	0.44 ± 0.33 ^a	38.15 ± 9.74 ^a	26.05 ± 8.93 ^a
TG	0.32 ± 0.12 ^{ab}	3.27 ± 0.20 ^b	25.99 ± 1.55 ^a	3.15 ± 0.50 ^a	0.74 ± 0.17 ^a	33.38 ± 21.47 ^a	23.51 ± 22.35 ^a
TDT	0.38 ± 0.03 ^a	6.34 ± 1.02 ^a	37.14 ± 0.81 ^a	3.17 ± 0.62 ^a	0.55 ± 0.44 ^a	43.15 ± 13.05 ^a	28.87 ± 8.09 ^a
TNG	0.24 ± 0.06 ^{ab}	3.28 ± 0.14 ^b	32.37 ± 1.01 ^a	3.10 ± 0.43 ^a	0.44 ± 0.27 ^a	55.04 ± 0.12 ^a	43.17 ± 1.84 ^a
Norm (Harkness et al. 2010)	0.366-0.6	3.1-3.164	-	1.17- 8.775	-	54-269	27-77

S20M80: *soumbara* 20 % and *Moringa* leaf powder 80 %; S30M70: *soumbara* 30 % and *Moringa* leaf powder 70 %; S50M50: *soumbara* 50 % and *Moringa* leaf powder 50 %; S70M30: *soumbara* 70 % and *Moringa* leaf powder 30 %; S80M20: *Soumbara* 80 % and *Moringa* leaf powder 20 %; S: *soumbara* ; M: *Moringa* leaf powder; TG: diabetic control treated with glibenclamide; TDT: untreated diabetic control; TNG: normoglycemic control; TB: Total bilirubin; CB: Conjugated bilirubin. Values are expressed as mean ± standard deviation for three independent measurements. Mean values with a different letter in a column are significantly different based on Tukey's test at the 0.05 threshold.



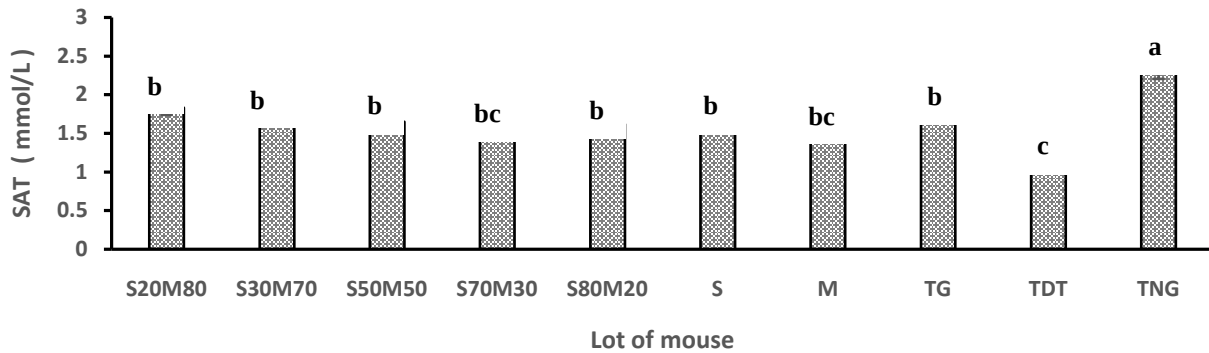
S20M80: *soumbara* 20 % and *Moringa* leaf powder 80 %; S30M70: *soumbara* 30 % and *Moringa* leaf powder 70 %; S50M50: *soumbara* 50 % and *Moringa* leaf powder 50 %; S70M30: *soumbara* 70 % and *Moringa* leaf powder 30 %; S80M20 : *Soumbara* 80 % and *Moringa* leaf powder 20 %; S: *soumbara* ; M: *Moringa* leaf powder; TG: diabetic control treated with glibenclamide; TDT: untreated diabetic control; TNG: normoglycemic control. Values are expressed as mean ± standard deviation for three independent measurements. Mean values with a different letter in a column are significantly different based on Tukey's test at the 0.05 threshold.

Figure 3. Effects of formulations on mouse superoxide dismutase (SOD)



S20M80: *soumbara* 20 % and *Moringa* leaf powder 80 %; S30M70: *soumbara* 30 % and *Moringa* leaf powder 70 %; S50M50: *soumbara* 50 % and *Moringa* leaf powder 50 %; S70M30: *soumbara* 70 % and *Moringa* leaf powder 30 %; S80M20 : *Soumbara* 80 % and *Moringa* leaf powder 20 %; S: *soumbara* ; M: *Moringa* leaf powder; TG: diabetic control treated with glibenclamide; TDT: untreated diabetic control; TNG: normoglycemic control. Values are expressed as mean ± standard deviation for three independent measurements. Mean values with a different letter in a column are significantly different based on Tukey's test at the 0.05 threshold.

Figure 4. Effects of formulations on mouse glutathione peroxidase (GPx)



S20M80: *soumbara* 20 % and *Moringa* leaf powder 80 %; S30M70: *soumbara* 30 % and *Moringa* leaf powder 70 %; S50M50: *soumbara* 50 % and *Moringa* leaf powder 50 %; S70M30: *soumbara* 70 % and *Moringa* leaf powder 30 %; S80M20: *Soumbara* 80 % and *Moringa* leaf powder 20 %; S: *soumbara*; M: *Moringa* leaf powder; TG: diabetic control treated with glibenclamide; TDT: untreated diabetic control; TNG: normoglycemic control. Values are expressed as mean $\pm$ standard deviation for three independent measurements. Mean values with a different letter in a column are significantly different based on Tukey's test at the 0.05 threshold.

Figure 5. Effects of formulations on antioxidant status of mouse

3.8. Effects of Formulations on Mouse Organ Biometry

The effect of formulations on the average organ weights of mouse is recorded in Table 5. Analysis of the results showed a significant difference ($p < 0.05$) in all organs except the heart. Mean lung and spleen weights were higher in the glibenclamide-treated control (1.31 ± 0.32 g and 1.15 ± 0.14 g respectively) than in the other batches of mice, which were statistically identical. In terms of mean kidney weight, mouse on the S30M70 diet and the untreated diabetic control had a high mean weight of 2.58 ± 0.05 g and 2.56 ± 0.57 g respectively, whereas the normoglycemic control had a low mean weight (1.07 ± 0.04 g). As for the liver, the average weight was high in

mouse fed *Moringa* leaves (7.83 ± 0.83 g) and lower in the normoglycemic control (3.89 ± 0.20 g).

Biometric analysis of the mice's organs was used to assess the risks associated with consumption of the different diets. Biometrics showed that the average weights of the kidneys, spleen, lungs and liver increased significantly ($p < 0.05$) compared with those of the normoglycemic control. However, the mean organ weights of mouse on the formulated diets were broadly identical to those of the untreated diabetic control and the glibenclamide-treated control. This increase in organ weight is thought to be due, on the one hand, to the metabolic disorder caused by alloxane and, on the other, to the hyperactivity imposed by substances that are difficult to metabolize, filter and excrete excess metabolic waste products [44].

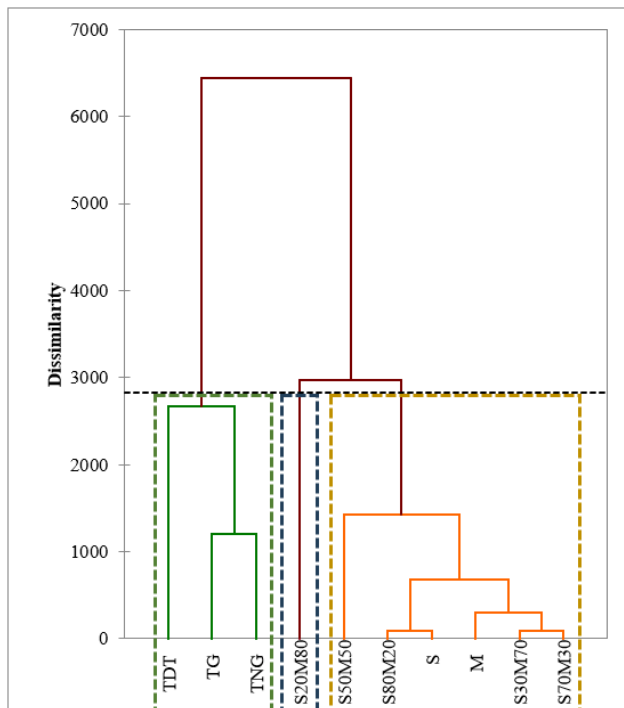
Table 5. Effects of formulations on organ biometry in mice

Lot of mouse	Organs				
	Heart	Lungs	Foie	Spleen	Kidney
S20M80	0.63 ± 0.07^a	1.07 ± 0.07^b	6.79 ± 0.60^{abc}	0.59 ± 0.06^{ab}	1.98 ± 0.24^{ab}
S30M70	0.52 ± 0^a	1.45 ± 0.05^b	7.30 ± 0.05^{ab}	0.48 ± 0.02^b	2.58 ± 0.05^a
S50M50	0.57 ± 0.04^a	0.87 ± 0.04^b	5.17 ± 0.19^{de}	0.37 ± 0^b	2.16 ± 0.04^{ab}
S70M30	0.55 ± 0.06^a	1.03 ± 0.20^b	7.22 ± 0.09^{ab}	0.44 ± 0.04^b	1.92 ± 0.23^{ab}
S80M20	0.48 ± 0.06^a	1 ± 0^b	5.04 ± 0.33^b	0.41 ± 0.06^b	1.58 ± 0.26^{bc}
S	0.47 ± 0.04^a	0.79 ± 0.13^b	6.38 ± 0.71^{bcd}	0.76 ± 0.32^{ab}	2.04 ± 0.36^{ab}
M	0.60 ± 0.08^a	1.23 ± 0.07^b	7.83 ± 0.83^a	0.54 ± 0.07^{ab}	2.25 ± 0.39^{ab}
TG	0.42 ± 0.13^a	1.31 ± 0.32^a	5.53 ± 1.44^{cd}	1.15 ± 0.14^a	2.2 ± 0.20^{ab}
TDT	0.63 ± 0.11^a	1.07 ± 0.12^b	5.64 ± 1.34^{cd}	0.69 ± 0.29^{ab}	2.56 ± 0.57^a
TNG	0.31 ± 0.02^a	0.56 ± 0.03^b	3.89 ± 0.20^e	0.28 ± 0.02^b	1.07 ± 0.04^c

S20M80: *soumbara* 20 % and *Moringa* leaf powder 80 %; S30M70: *soumbara* 30 % and *Moringa* leaf powder 70 %; S50M50: *soumbara* 50 % and *Moringa* leaf powder 50 %; S70M30: *soumbara* 70 % and *Moringa* leaf powder 30 %; S80M20: *soumbara* 80 % and *Moringa* leaf powder 20 %; S: *soumbara*; M: *Moringa* leaf powder; TG: diabetic control treated with glibenclamide; TDT: untreated diabetic control; TNG: normoglycemic control. Values are expressed as an average $\pm$ standard deviation for three independent measures. The mean values with a different letter in a column are significantly different based on the Tukey test at the 0.05 threshold.

3.9. Cluster Dendrogram of Formulations

This analysis showed that from 25 parameters analyzed (Weight gain, blood glucose, total cholesterol, phosphorus, sodium, iron, calcium, potassium, triglycerides, HDL, urea, uric acid, creatinine, total bilirubin, conjugated bilirubin, AST, ALT, heart, lungs, liver, spleen, kidneys, GPX, SOD, SAT.) point view, three groups distinct of formulations have been revealed. The first group was composed only formulation S20M80 while the second groupe included formulations follows: S30M70 ; S50M50 ; S70M30 ; S80M20 ; S ; M and the last group was constituted of formulations TG, TDT, TNG (Figure 6).



S20M80: *soumbara* 20 % and *Moringa* leaf powder 80 %; S30M70: *soumbara* 30 % and *Moringa* leaf powder 70 %; S50M50: *soumbara* 50 % and *Moringa* leaf powder 50 %; S70M30: *soumbara* 70 % and *Moringa* leaf powder 30 %; S80M20: *soumbara* 80 % and *Moringa* leaf powder 20 %; S: *soumbara*; M: *Moringa* leaf powder; TG: diabetic control treated with glibenclamide; TDT: untreated diabetic control; TNG: normoglycemic control. Values are expressed as an average $\pm$ standard deviation for three independent measures. The mean values with a different letter in a column are significantly different based on the Tukey test at the 0.05 threshold.

Figure 6. Cluster dendrogram of formulations

4. Conclusion

Daily administration of aqueous extracts from formulated *soumbara* and *Moringa* leaf diets, notably formulations S20M80 and S30M70, to mouse by gavage resulted in a significant reduction in hyperglycemia and weight gain in diabetic mice. Analysis of serum biochemical parameters showed that the formulated diets did not adversely affect the lipid profile of the mice. However, an increase in serum iron and electrolyte levels was observed in mouse fed the formulated diets, compared with the various control batches. Finally, the results revealed a significant decrease in total antioxidant status,

superoxide dismutase activity and erythrocyte glutathione peroxidase in diabetic mouse compared to the normoglycemic control. Thus, formulated foods and in particular the S20M80 formulation could be recommended to people affected by diabetes and iron-deficiency anemia.

Conflict of interest statement

Authors declare that they have no conflict of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethics approval

The work research complies with the current animal welfare laws in Ivory Coast. The provisions of the Govt. of Ivorian's Wildlife Protection Act of 1965 are not applicable for experiments on this mouse. All experimental protocols were approved by the University Nangui Abrogoua ethics committee.

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