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Antidiabetic and safety evaluation of Afya tea® (Aqueous extract of *Moringa oleifera* Lam.) in streptozotocin-rat model

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Abstract

Objective: The present work was set to evaluate the *in vivo* efficacy and safety of "Afya tea®", a trademarked antidiabetic herbal preparation commonly sold in Cameroon.

Methods: The tea was acquired from local sale points and different concentrations and doses determined from the prescription by the manufacturer. The antidiabetic activity was assessed in wistar albino rats using both the oral glucose tolerance test (OGTT) and the subacute antidiabetic assay in streptozotocin-rat model. The safety was evaluated combining the *in vitro* (on LLC-MK2 Monkey kidney epithelial cell line) and *in vivo* acute toxicity (in mouse model) tests. The effects of Afya tea® were observed on fasting blood sugar (FBS), body weight, water and food intake, urine elimination, selected markers of liver and kidney functions, as well as lipid profile.

Results: The OGTT revealed a significant drop in blood glucose levels in rats treated with 84.6 g/kg Afya tea®, as compared to the control; together with drop in critical peak of blood sugar. The therapeutic effect observed was equally more consistent with the dose of 84.6 g/kg , resulting in significant recovery from STZ-induced diabetes in animals as reflected in the FBS level, body weight, food and water intake and urine elimination.

Conclusion: The present work confirmed the positive effect of Afya tea® on glucose tolerance, its subacute antidiabetic potential of this herbal preparation in sptreptozotocin-induced diabetic rats, as well as its relative safety. Further studies including the study of the stability pattern and mechanism of action as well as clinical trials are warmly expected, to fully appreciate the suitability of this herbal preparation for treatment of diabetes mellitus in human.

Keywords: Afya tea®, *Moringa oleifera*, Diabetes

Introduction

Diabetes mellitus (DM) is a chronic and complex metabolic dysfunction characterized by persistent high blood sugar as a result of defective insulin secretion and/or poor use of this hormone by the system. It is one of the largest health emergencies of the 21st century and counts among the leading causes of death and disability worldwide, with an estimated global prevalence of 8.8% which is predicted to reach 10% by 2030 with a projected 642 million people affected by 2040 [1]. World Health Organization (WHO) report for 2016 highlights that diabetes is on the rise and has moved from a disease of predominantly rich nations, to become a steadily growing health issue everywhere, most markedly in the world's middle-income countries. The situation is worsened in developing countries by "the lack of effective policies to create supportive environments for healthy lifestyles coupled with limited access to quality health care, particularly for poor people [2]. In the 7th edition of its diabetes Atlas, 2015, international diabetes federation (IDF) estimated that 1 in 11 adults had diabetes and that 1 in 2 with diabetes was not diagnosed and up to 542,000 children have type 1 diabetes [1]. Nearly 80% of people living with diabetes are found in low-and middle income countries [3, 4], though Africa has the lowest prevalence (3.4%) compared to other continents [1]. In 2010, IDF estimated the Cameroon prevalence of diabetes among adults aged 20 to 79 years at 4.4%. Prevalent undiagnosed diabetes in Cameroon is also very high, about 80% [5].

Generally, controlling blood sugar levels is crucial for diabetes management, in order to avoid or delay the upset of complications. While Type 1 diabetes is managed by insulin injections, or can be completely treated with pancreas

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transplantation, Type 2 is more challenging. Most medications for type 2 diabetes are oral drugs. Some people with this form of diabetes may also need to take insulin. Unfortunately reverting the type 2 diabetes condition remains an enormous challenge and no single medication is free of adverse effects. The need for new generation of antidiabetics thus remains an urgent concern and natural products represent a promising, yet fully explored source of new therapies. *Moringa oleifera* Lam (Moringaceae) is a multipurpose medicinal plant which has been regarded as a food substance since ancient times and has also been used as a treatment for many diseases [6]. The therapeutic potential of various parts of this plant has been extensively investigated and phytomedicines do exist from this plant in several forms. Different usages of the plant species as antimicrobial, antioxidant, anticancer, anti-inflammatory and antidiabetic have been reported, with more or less sustaining scientific evidence [6-10]. In a society with growing burden of diabetes with all the health challenges posed by the disease, "Afya tea®" was thus conceived by ETS AFYA TEA, a home-grown Cameroonian company. The design of this herbal preparation was probably inspired by the acclaimed virtues of *Moringa oleifera* locally and internationally. This tea is produced directly from the leaves of Moringa, which grows easily in Cameroon, particularly in humid forest where leaves are abundantly produced yearly from volcanic soils of Buea, Kumba and Douala vicinities. As prescribed by the manufacturing company, patients are advised to use Afya tea® by boiling 2 teaspoons of tea per teacup of water, for an average adult. From the producers and sellers, Afya tea® is thought to reduce blood glucose in diabetic patients as well as alleviating other signs and symptoms of the diseases such as abundant polyuria, polydipsia, weight loss and fatigue. Reportedly, no side effect had been recorded by patients under treatment. However, it is strongly recommended that patients should eat before taking the tea, if not, some effects like dizziness and tiredness can occur.

In order to investigate the actual antidiabetic potential of Afya tea®, the present work was therefore prompted, aiming at assessing the acute hypoglycaemic properties and the sb-acute anti-diabetic effects of the tea, in streptozocin-induced diabetic rats, as well as its safety.

Materials and methods

Biological material, chemicals and reagents

The tea was acquired from local sale points and different concentrations and doses determined from the prescription by the manufacturer. Streptozotocin, glibenclamide, glucose, chloroform, culture media and supplements (RPMI-1640, L-glutamine, penicillin, streptomycin, newborn calf serum) and other laboratory consumables were purchased from Sigma-Aldrich (Germany). The different kits for biochemical parameters (total cholesterol, triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (gamma GT), creatinine, uric acid, glucose) were purchased from ChronoLab® (Barcelona,Spain). The Monkey kidney epithelial Cell line (LLC-MK2) was obtained from the American Type Culture Collection (ATCC), Virginia, USA.

Experimental animals

The experimental animals (wistar albino rats and Balb/c mice) were provided by the animal breeding unit of the medical research and applied biochemistry laboratory, university of Buea. Animals were handled according to the ethical guidelines of the Cameroon national veterinary laboratory (LANVET, Ministry of livestock, fisheries and animal industry) and the ARRIVE (Animal research: Reporting *in vivo* experiments) guidelines.

Dosage determination and extract preparation

The company producing the Afya tea® recommends that 2 teaspoon of the dry tea be used in one teacup of water and processed using a coffee machine or any other heater. An adult patient is advised to take this twice per day. The estimation of the quantity of dry matter corresponding to 2 teaspoon was determined as 5.25±0.31g, from 10 repeats of measurement and weighing using a high-precision electronic balance (Fa-2104B, Joan Lab®, China). The average weight per sachet was equally determined: 52.54 g. The average volume of tea cup was considered to be 180 mL. Based on the above findings, approximations were made to have 5 g of the tea suspended into 200 mL of distilled water (for the standard dose, named Afya tea® X) and submitted to 5 min boiling at 100 °C in a water bath (Victor Recker Model GFL1083). The extract was then allowed to get cold before filtration. From the initial 200 mL, an average volume of 165 mL of tea (water extract) was obtained and the average extract concentration was found to be 12 mg dry matter per ml tea for the highest dose tested, namely Afya tea® 3X. The exploitation of these data led to the following dosage: 7.05 mL per kg body weight (84.6 mg per kg body weight, Afya tea® 3X). The other doses (Afya tea® 2X, Afya tea® X/2, and Afya tea® X/3) were thereafter derived from the Afya tea® 3X by dilution in distilled water. It is worth mentioning that the Afya tea® samples tested in this study were randomly collected from the local sale points in Buea, Cameroon.

Oral glucose tolerance test (OGTT)

Diabetes mellitus always arises as a result of failure in the organism to regulate blood glucose levels. Normally, blood glucose levels will rise after a meal, as a result of absorption of glucose from the meal.

This transitional hyperglycemia is reversed by regulatory mechanisms of the organism generally within 2-4 hrs. The main objective of OGTT was to investigate the ability of Afya tea® to stimulate regulations/reduction of blood sugar level, in non-diabetic subjects. A total of 58 (male and female) wistar

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albino rats were divided into different (11) groups of 5-6 each according to body weight (Table1).

The OGTT was carried out following the method previously described [11, 12]. In brief, the animals were fasted overnight prior to commencement of the test. A drop of blood sample was collected from the tail of each animal and applied on strip of a glucometer (CodeFree®, SD Biosensor, South Korea) for glucose measurement. Immediately, the animal was orally administered corresponding product (tea extract, glibenclamide, or distilled water), and the time recorded as-30 min. Blood glucose levels of the animals was also measured at 0 min (i.e. at-30 min)and the animals loaded with an oral dose of glucose (2 g/kg bwt). The level of glucose in the animal's blood was further recorded at 30 min, 60 min, 90 min, 120 min and 240 min. It should be noted that animals from groups 3 and 7 were not given glucose and enabled assessment of the effect of the tea on blood glucose levels when the tea is taken by individuals in a fasting state.

Table1: Different groups of animals used for oral glucose tolerance test

Sex	Group	Number of animals	Treatment
Female	1	5	Glibenclamide (3 mg/kgbwt) + glucose (2 g/kgbwt)
	2	6	Glucose (2 g/kg bwt)
	3	6	Afya tea® 3X (84.6 mg/kg)
	4	6	Afya tea® 3X (84.6 mg/kg) + glucose (2 g/kg bwt)
Male	5	5	Glibenclamide (3 mg/kg bwt) + glucose (2 g/kg bwt)
	6	5	H_2O + glucose (2 g/kg bwt)
	7	5	Afya tea® 3X (84.6 mg/kg)
	8	5	Afya tea® 3X (84.6 mg/kg) + glucose(2 g/kg bwt)
	9	5	Afya tea® X/2(14.1 mg/kg) + glucose(2 g/kg bwt)
	10	5	Afya tea® X/3 (9.4 mg/kg) + glucose(2 g/kg bwt)
	11	5	Afya tea® X (28.2 mg/kg) + glucose(2 g/kg bwt)

"X" is the normal dosage prescribed by ETS. AFYA TEA®: 2 teaspoon of dry tea in 1 teacup for a human adult (average body weight of 70 kg). The dosage range was more extended in males than females, given the higher susceptibility of this sex to diabetes.

Antidiabetic activity effect of the tea in diabetic experimental rats

The sub-acute antidiabetic activity of the different doses was determined in rats with streptozotocin-induced diabetes, following the method of Al-Shamaony et al. [13]. Male and female rats of 12-week-old, with 135-327 g body weight, were submitted to 12 h fasting prior to diabetes induction. Each of the animals subsequently received an intravenous injection of 50 mg/kg dissolved in 0.1 M citrate buffer at pH 4.5. After 3 days, the animals with at least 200 mg/dL fasting blood sugar (FBS) were considered "diabetic" and used for the assays.

A total of 50 (22 males, 28 females) diabetic animals were divided into 6 groups, of 4-5 males and 4-5 females each and having comparable average body weight. The groups were assigned different treatments as follows: reference drug (glibenclamide 3 mg/kg/day), control (distilled water), Afya tea® X, Afya tea® 2X, Afya tea® 3X, Afya tea® X/3. It should be noted that 2 groups of 5 non-diabetic rats were also included and treated with distilled water and 3 time recommended dose of the tea (Normal Afya tea® 3X) respectively.

The different groups were receiving food and water *ad libitum* but fasting overnight before FBS measurement. The different treatments were administered daily, for 14 consecutive days and FBS measured on days 0 (before drug administration), day 7 and 14 (end of the treatment period) following the same protocol as for OGTT. The animal's body weight, food and water intake, volume of urine eliminated were also evaluated throughout the treatment and the animals monitored for any behavioral change.

At the end of the treatment period, the rats were sacrificed, blood collected to prepare serum samples, which was subsequently used to determine the glucose, lipid profile (total cholesterol, triglycerides), markers of liver ALT, AST, ALP and gamma GT activities and kidney (creatinine, uric acid levels) function.

Safety assessment of the tea

Verification was done both *in vitro* and *in vivo.* The tea extract was dried at 50 °C and the residue used for the assays.

In vitro cytotoxicity evaluation

Monkey kidney epithelial cells were distributed in 96 -well plates at 22,000 cells in 150 µL culture medium (RPMI-1640 medium with supplemented with L-glutamine, penicillin, streptomycin, and 10% newborn calf serum) per well and incubated at 37 °C under 5% CO_2 in humidified air to enable attachment. After 24 hrs of incubation, different concentrations of the aqueous extract were added into the cultures to achieve a final concentration range of 7.8-1000 µg/ml (1000, 500, 250, 125, 62.5, 31.3, 15.6 and 7.8 µg/mL). Negative control wells were incubated with culture medium only. The culture plates were then incubated for 72 hrs at 37 °C under 5% CO_2 in humidified air and MTT assay conducted

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as reported earlier [14]. The optical densities were recorded at 595 nm and viability in each well calculated using the formula:

$$\text{Viability}(\%) = [\text{Absorbance Test} / \text{Absorbance Control}] \times 100$$

In vivo acute toxicity evaluation

The acute toxicity of the tea was assessed using both adult males and females Balb/C mice, according to Organization for Economic Cooperation and Development (OECD) guidelines for testing of chemicals [15]. Healthy young adult male and female mice were used. In brief, the animals of each sex were randomly divided into 2 groups of 5 each, making a total of 4 groups which were kept in their respective cages for 5 day acclimatization. For each sex, animals of one group (test group) received the plant extract at a fixed dose of 2000 mg/kg, while the other animals (control group) were administered the vehicle (distilled water).The extract and vehicle were administered orally using a feeding needle. It should be noted that the animals were fasted 4 hrs prior to dosing and 2 hrs after dosing with extract or vehicle, while water was made available throughout the experiment. The mice were observed periodically for 4 hrs following administration of tea/vehicle and every day for 14 days for detection of any behavioral alterations. Feed and water consumption were monitored. Body weight was also recorded on days 7 and 14.

Results and discussion

Oral glucose tolerance test (OGTT)

The acute hypoglycemic effect of Afya tea was investigated in rats and results presented in Tables 2 and Figure 1.

All the groups for the OGT were statistically similar at the beginning for the body weight (Duncan, p=0.139) and also homogeneous for FBS at the time point 30 min, i.e., before treatment. As shown by the results, significant differences in blood glucose levels were observed across groups at any given point of time (P>0.01), except for time 120 min (P=0.135). A slight but not statistically significant drop was observed with the highest dose of the tea (Afya tea® 3X, corresponding to 6 teaspoons of powder in 3 teacup of water for a human adult), 30 minutes post-administration (Time point 0 minute). However, comparison between males and females revealed a more pronounced acute hypoglycemic effect of Afya tea in males than female animals when administered at this dosage (ANOVA Duncan, p<0.05).The ability of the tea to reduce blood sugar level, particularly at Afya tea® 3X, (84.6 mg/kg), is confirmed by the low blood sugar peak observed upon administration of high concentration of glucose to the rats (Time point 30 min).

Table 2a: Variation of blood glucose levels in female rats during OGTT

Group	Treatment	Body weight	Blood glucose (mg/dL)						
			-30 min	0 min	30 min	60 min	90 min	120 min	240 min
1	Glibenclamide (3 mg/kgbwt) + Glucose (2 g/kg bwt)	184.72 ± 3.46	84.4 ± 8.9^{a}	101.0 ± 4.7^{a}	169.8 ± 7.7^{a}	146.8 ± 8.1^{a}	127.6 ± 6.8^{a}	96.6 ± 6.9^{a}	66.4 ± 4.4^{a}
2	Glucose(2 g/kg bwt)	219.70 ± 37.11	95.4 ± 11.2^{a}	91.8 ± 7.5^{ab}	149.8 ± 20.4^{bc}	139.8 ± 23.2^{bc}	123.0 ± 20.8^{a}	95.8 ± 8.9^{ab}	87.0 ± 8.9^{b}
3	Afya Tea® 3X	195.92 ± 42.84	87.6 ± 14.4^{a}	83.4 ± 9.4^{b}	95.0 ± 10.5^{c}	87.2 ± 13.8^{c}	89.2 ± 13.8^{b}	79.2 ± 6.8^{b}	65.0 ± 7.0^{a}
4	Afya Tea® 3X+glucose (2 g/kg bwt)	200.42 ± 32.72	86.4 ± 6.1^{a}	82.2 ± 4.2^{b}	135.2 ± 14.8^{b}	114.7 ± 24.0^{c}	94.6 ± 17.7^{b}	80.6 ± 16.3^{b}	77.0 ± 11.5^{ab}

Values presented are Mean ± SD of 5-6 animals per group. In each column, values with the same letters are statistically equal to each other (Waller-Duncan). "X" is the normal dosage prescribed by the manufacturer: 2 teaspoon of dry tea in 1 teacup for a human adult (average body weight of 70 kg).

Sub acute anti diabetic activity effect of the tea in diabetic experimental rats

The findings on the sub-acute antidiabetic effects of the different treatments are presented based on evolution in body weight, food and water intake, urine elimination, FBS, lipid profile as well as the markers of liver and kidney functions.

Effect of Afya tea® on body weight

A slight weight loss was observed in negative control and those of the animals receiving lower doses of Afya tea®, whereas minor increase was noticed with 3-fold the recommended dose, the positive control and all the non-diabetic rats (Figure 2). However, these changes were not statistically significant (p>0.05).

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Table 2b: Variation of blood glucose levels in different treatments in male rats during OGTT

Group	Treatment	Body weight	Blood glucose(mg/dL)						
			- 30 min	0 min	30 min	60 min	90 min	120 min	240 min
1	Glib + glucose (2 g/kg bwt)	274.34 ±13.91	82.8 ± 8.2^{a}	83.4 ± 5.0^{a}	134.4 ±6.9^{bc}	117.6 ± 20.1^{a}	102.2 ± 12.7	88.0 ± 9.4^{ab}	61.2 ± 6.5^{a}
2	H_2O + glucose (2 g/kg bwt)	198.90 ± 80.59	77.2 ± 9.0^{a}	83.7 ± 12.7^{a}	145.2 ± 20.5^{bc}	126.2 ± 10.1^{a}	111.8 ± 7.2^{b}	94.7 ± 10.9^{ab}	85.8 ± 10.0^{bc}
3	Afya tea® 3X	207.92 ± 68.34	81.0 ± 17.0^{a}	75.8 ± 11.7^{a}	20.5 ± 10.5^{a}	85.5 ± 11.5^{a}	87.2 ± 15.1^{a}	85.0 ± 18.8^{a}	70.7 ± 15.4^{ab}
4	Afya tea® 3X + glucose (2 g/kg bwt)	202.27 ± 61.97	84.2 ± 18.7^{a}	75.3 ± 17.4^{a}	129.3 ± 16.6^{bc}	140.5 ± 22.0^{a}	121.8 ± 20.6^{bc}	106.0 ± 11.5^{b}	94.3 ± 12.8^{d}
5	Afya tea® X/2 + glucose (2 g/kg bwt)	296.76 ± 38.80	79.4 ± 6.1^{a}	80.2 ± 9.0^{a}	172.4 ± 10.5^{d}	144.4 ± 4.3^{a}	122.4 ± 4.0^{bc}	97.4 ± 10.3^{ab}	87.8 ± 9.2^{c}
6	Afya tea® X/3 + glucose (2 g/kg bwt)	250.14 ± 45.64	75.2 ± 9.3^{a}	70.6 ± 6.2^{a}	178.0 ± 21.3^{d}	163.3 ± 14.8^{b}	136.8 ± 7.6^{c}	92.4 ± 8.9^{ab}	83.0 ± 7. 8^{bc}
7	Afya tea® X + glucose (2 g/kg bwt)	264.42 ± 13.51	75.4 ± 2.1^{a}	76.4 ± 5.0	157.2 ± 15.7^{cd}	122.8 ± 18.3^{a}	104.4 ± 15.5^{bc}	83.8 ± 6.8^{a}	79.6 ± 5.6^{bc}

Values presented are Mean ± SD of 5-6 animals per group. In each column, values with the same letters are statistically equal to each other (Waller-Duncan). "X" is the normal dosage prescribed by the manufacturer: 2 teaspoon of dry tea in 1 teacup for a human adult (average body weight of 70 kg).

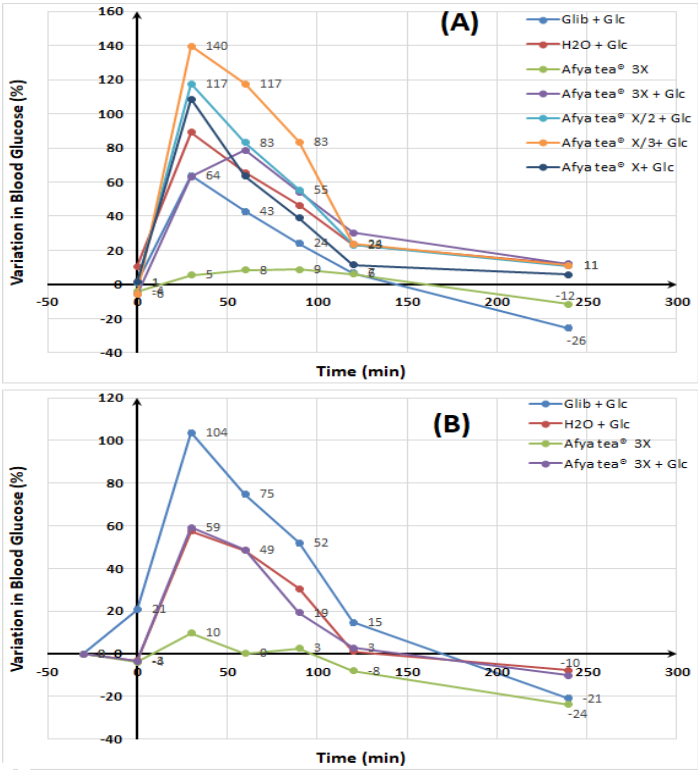

Figure 1: Variation of FBS in different treatments during oral glucose tolerance test in male (A) and female (B) rats

Animals were treated with different products/vehicle (either with glibenclamide (3 mg/kg), distilled water, or different doses of Afya tea) at 30 min and oral glucose load (2 g/kg) at 0 min. The blood glucose level was monitored from -30 to 240 min. It should be noted that two groups treated with 3X tea were not given glucose. The glucose levels were expressed in percentage relative to the baseline value, i.e., glycaemia at -30min.

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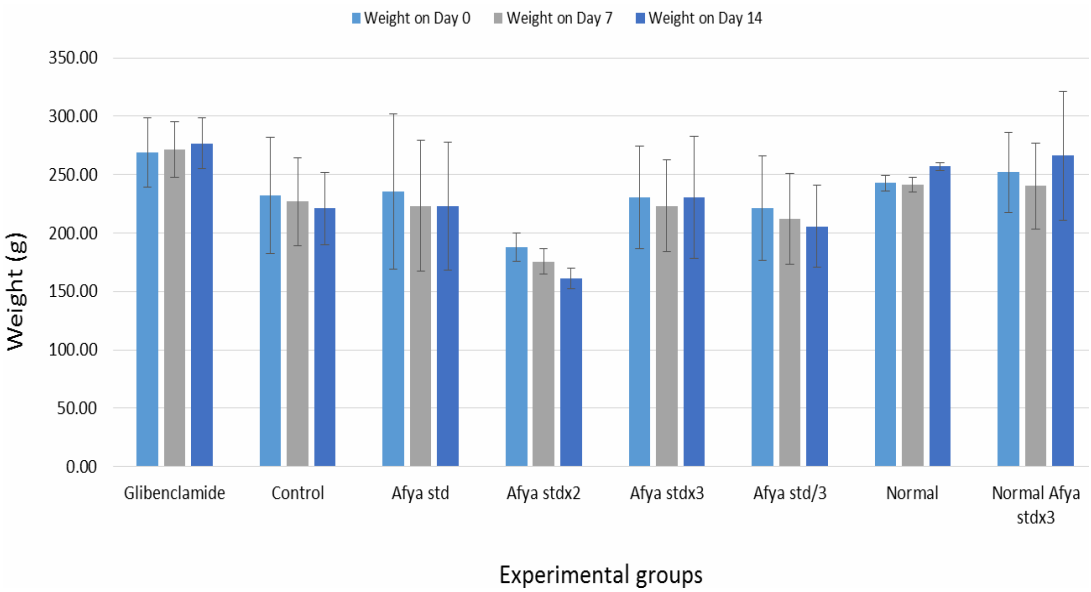

Figure 2: Effect of Afya tea® on body weight of diabetic and healthy rats

Values presented are Mean ± Standard deviations of 8-10 repeats including males and females animals. Afya std (Afya tea® X), Afya std 2X (Afya tea® 2X); Afya std 3X (Afya tea® 3X); Afya std X/3 (Afya tea® X/3), Control (distilled water), Glib (glibenclamide 3 mg/kg), Normal (non diabetic, distilled water) and Normal std X3 (Non diabetic rats receiving 3 times recommended dose).

Effect of Afya tea® on food and water intake and urine elimination

In general food intake by diabetic rats was significantly higher than that of the non-diabetic animals. However, in virtually all the treated groups as well as the control, a gradual decrease was noticed though more pronounced in animals receiving glibenclamide and the highest doses of the tea.

Similarly, the water intake of the diabetic animals was significantly high compared to that of the non-diabetic groups, followed by the positive control. However, no statistically important difference was observed among the treated groups. The urine elimination followed the same trend, but with a strong correlation between the volume of urine and the FBS level ($P < 0.01$).

Effect of Afya tea® on fasting blood sugar (FBS)

From figure 3, it appears that streptozotocin-induced diabetes may be self-resolving in wistar albino rats, as shown by the decrease in FBS in the control group. The predicted effect of the positive control (glibenclamide) is also confirmed by this experiment after 14 day treatment. Interestingly statistical analysis of the data obtained (One-way ANOVA, Duncan test) reveals that FBS level in glibenclamide-treated animals was similar to that of animals receiving 3-fold the standard dose of Afya tea® (84.6 mg/kg dry extract per body weight).

Effect of Afya tea® on liver and kidney functions and lipid profile

Figure 4 illustrates the total cholesterol and triglyceride levels of the experimental animals treated either with tea extract, glibenclamide or the vechile for 14 days. Except for the high cholesterol levels in the glibenclamide group and the high triglyceride levels in rats treated with 2-fold Afya tea® standard dose, all the animals showed lipid profiles within the normal ranges. There was no significant difference among groups which may be attributed to the type of treatment. Also, there was no statistically significant different among the experimental groups as the markers of liver and kidney functions (ALT, AST, ALP and creatinine) were concerned (data not shown).

Acute toxicity in mice

Figure 5 shows the variation in body weight of male and female mice treated with high dose of Afya tea® (2000 mg/kg) and the controls. Overall, there was increase in body weight of the animals, including those receiving the tea, though no statistically significant difference was recorded between the control groups and the animals under treatment.

Cytotoxicity on LLC-MK2 cell line

As shown in Figure 6 incubation of cells with the Afya tea® aqueous extract did not induce any change in cell viability, even at the highest investigated concentration, 1000 µg/ml. Fifty percent inhibition wasn't achieved with the latter concentration, suggesting a cytotoxicity concentration 50 (CC50 concentration inducing 50% of cytotoxicity) higher than 1000 µg/ml. The extract can therefore be considered as safe for the LLC-MK2 cells, given that CC50 is 33 times higher than 30 µg/ml, a concentration that has been suggested as cut point for lack of cytotoxicity [16, 17].

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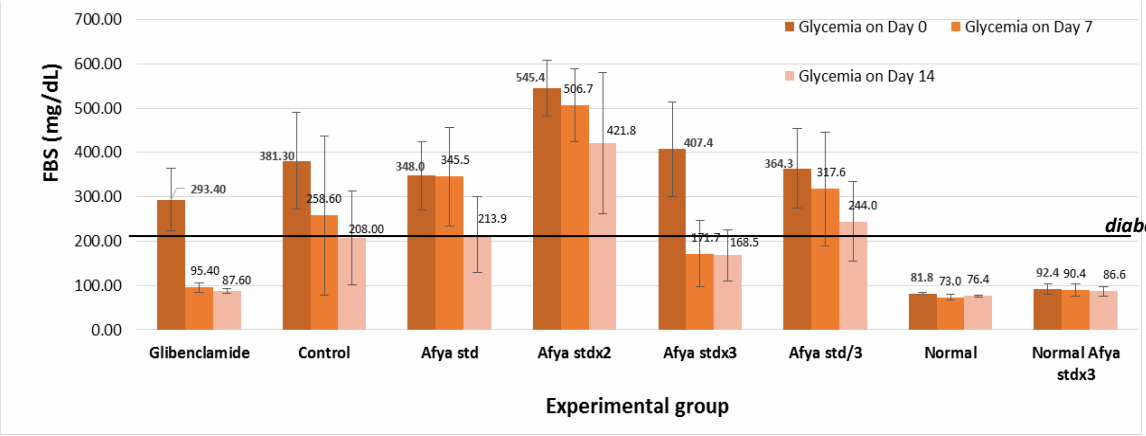

Figure 3: Effect of Afya tea® on FBS in diabetic and healthy rats

Values presented are Mean ± Standard deviations of 8-10 repeats including males and females animals. Afya std (Afya tea® X), Afya std 2X (Afya tea® 2X); Afya std 3X (Afya tea® 3X); Afya std X/3 (Afya tea® X/3), Control (distilled water), Glib (glibenclamide 3 mg/kg), Normal (non-diabetic, distilled water) and Normal std X3 (Non diabetic rats receiving 3 times recommended dose).

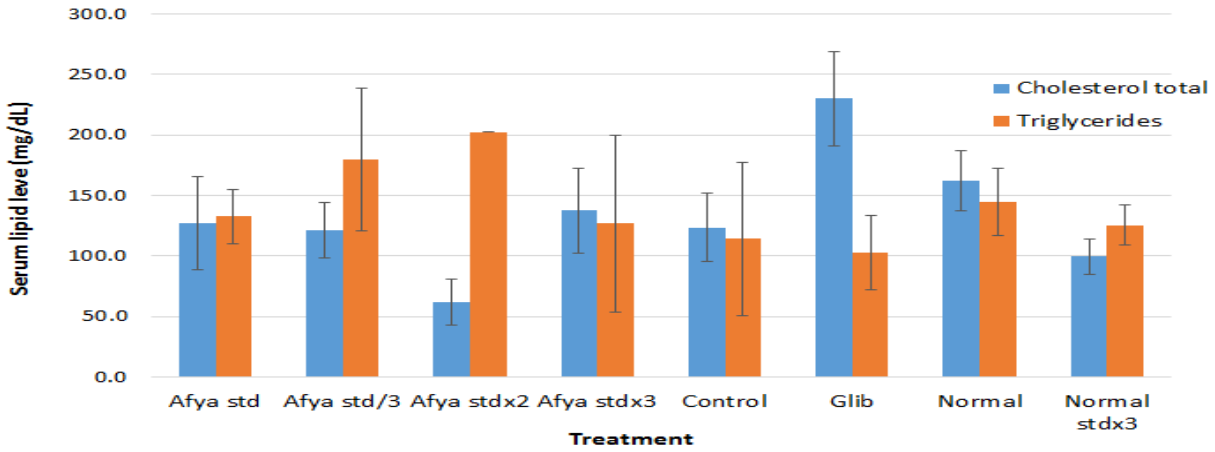

Figure 4: Effect of Afya tea® on serum lipid profile in diabetic and healthy rats

Values presented are Mean ± Standard deviations of 8-10 repeats including males and females animals. Afya std (Afya tea® X), Afya std 2X (Afya tea® 2X); Afya std 3X (Afya tea® 3X); Afya std X/3 (Afya tea® X/3), Control (distilled water), Glib (glibenclamide 3 mg/kg), Normal (non-diabetic, distilled water) and Normal std X3 (Non diabetic rats receiving 3 times recommended dose).

Toxicity studies

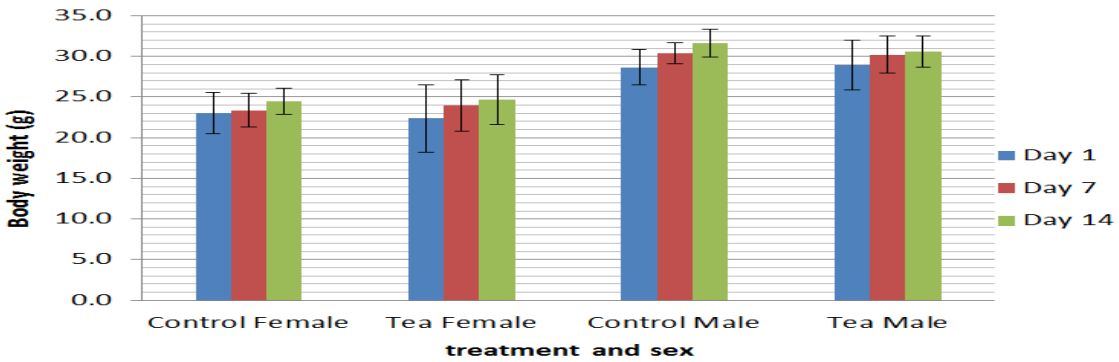

Figure 5: Body weight of the animals during 14 days follow-up period

Values presented are Mean ± Standard deviations of 5 repeats for each group.

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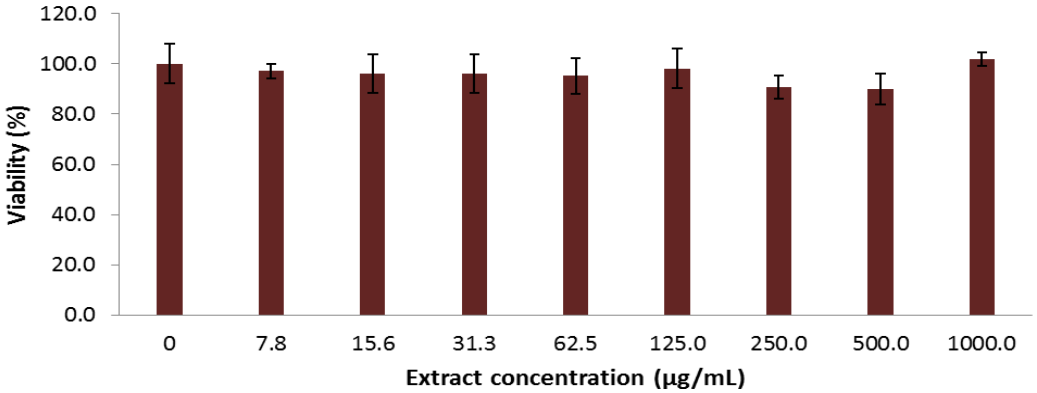

Figure 6: Viability of LLC-MK2 cells in the presence of different concentrations of Afya tea®

Values presented are Mean ± Standard deviations of 6 repeats from two separate experiments (in triplicate each).

M. oleifera commonly known as drumstick tree, horse radish tree or benzoil tree in most Asian countries or Moringa in Cameroon. It grows in several African countries, such as Benin, Burkina Faso, Cameroon, Chad, Eritrea, Ethiopia, Gambia, Ghana, Guinea, Kenya, Liberia, Mali, Mauritania, Niger, Nigeria, Senegal, Sierra Leone, Sudan, Tanzania, Togo, and Uganda [18]. Moringa is a multipurpose food and medicinal plant with enormous virtues. The fruit of the tree, very popular as a vegetable in Asia and African countries, is named as drumstick in India. The leaves are estimated to contain more Vitamin A compared to carrots, more calcium than milk, more iron than spinach, more Vitamin C than oranges, and more potassium than bananas. It is equally argued that the protein quality of Moringa leaves may rival that of milk and eggs [18]. It is reported that different parts of the Moringa trees have been used to tackle malnutrition, particularly among infants and nursing mothers [18-20] and Kuete [21] carried out an extensive review analysis and reported that various parts of this plant, such as the leaves, roots, seed, bark, fruit, flowers, and immature pods are employed as cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial, and antifungal activities. The seed extracts demonstrated significant activity on hepatic carcinogen metabolizing enzymes and skin papillomagenesis in mice [22].

The plant was also shown to possess ameliorative effects on lead-induced liver and kidney damages in wistar rats, suggesting the potential of the leave in treating lead poisoning and exposure to this major environmental pollutant [23]. Several bioactive molecules have been purified and characterized from Moringa preparations reported to exhibit hypotensive, anticancer and antimicrobial activities [24].

One of the major challenges faced by traditional pharmacopeia has been the difficulty to conserve the products in order to be able to export it out of the production site. Unfortunately pharmaceutical firms from the western world seem more interested in purified molecule drugs, rather than medicinal raw materials. With the growing epidemics of diabetes in Africa and Cameroon in particular, home-born initiatives are therefore highly encouraged. It is in this context that ETS AFYA TEA mainly focus to develop, produce and market an antidiabetic tea (Afya tea®) from the leaf of Moringa cultivated in Cameroon. The present work contributes at evaluating the therapeutic potential and safety of this product. The effects of Afya tea® were investigated using the following indicators: FBS, body weight, water and food intake, urine elimination, selected markers of liver and kidney functions as well as lipid profile. The OGTT revealed the ability of Afya tea® to acutely stimulate glucose intake and/or use by tissue, and even minimize the critical peak of FBS in healthy animals, as reflected in males by a 7.2% of FBS compared to 25% for glibenclamide, with reference to the initial glycaemia. The possible hypoglycemic effect observed was more pronounced in males than female rats, and the standard dose generally recommended by the manufacturer (28.2 g/kg) was not as efficient as the highest dose tested (84.6 mg/kg). This acute hypoglycemic effect of Afya tea® was later confirmed by significant recovery from STZ-induced diabetes in animals as reflected in the body weight, food and water intake and urine elimination. Considering the fact that polyphagia is one of the major characteristics of diabetes mellitus, a decrease in food intake that was also observed, might be interpreted as a result of a recovery from the disease in the animals being treated. Based on FBS, total recovery was achieved with the dose of 84.6 mg/kg and glybenclamide, highlighting the therapeutic potential of this herbal formulation. These observations suggest presence of bioactive compounds capable of lowering blood glucose levels. Indeed number of molecules such as 4-(4'-O-acetyl-α-Lrhamnopyranosyloxy) benzyl isothiocyanate, 4-(α-L-rhamnopyranosyloxy) benzyl

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isothiocyanate, niazimicin, pterygospermin, benzyl isothiocyanate, and 4-(α-L rhamnopyranosyloxy) benzyl glucosinolate have been isolated from Moringa [24]. Glucose-lowering effects of *M. oleifera* extracts have been previously reported. Olayaki et al [25] found that administration of methanol extract of the leaf significantly improved glucosetolerance and increased serum insulin levels. The authors concluded that hypoglycemic effects of *M. oleifera* extract might be mediated through the stimulation of insulin release leading to enhanced glucose uptake and glycogen synthesis. Significant drop in serum concentrations of triglyceride, total cholesterol and low-density lipoprotein (LDL)-cholesterol enhanced serum level of high-density lipoprotein was also reported [21, 25]. However, the present work didn't show any effect of Afya tea (water extract) on lipid profile in diabetic rats. Activities of ALT, AST and ALP in serum were not affected significantly by Afya tea, suggesting the absence of any hepatotoxicity induced by the tea. Likewise, the kidneys function was not affected, as illustrated by the levels of serum creatinine which remained unaltered.

The antidiabetic effect of *M. oleifera* seeds was reported on streptozotocin-induced diabetes and diabetic nephropathy in male rats [26]. Abd El Latif et al. [27] showed that aqueous extract from *M.oleifera* leaf exerts hypoglycemic effect in alloxan-induced diabetic rats, illustrated by normalized serum levels of glucose, triglycerides, cholesterol and malondiadehyde in serum. Another study conducted by Iffiu-Soltesz et al. [27] showed that chronic administration of benzylamine, a constituent of *M. oleifera*, in the drinking water could significantly improve glucose tolerance and reduce circulating cholesterol in high-fat dieted mice.

Acute toxicity revealed that both male and female animals receiving up to 2000 mg/kg Afya tea® did not exhibit any sign of toxicity in terms of mortality, food and water intake and change in behavior. Instead, animals under treatment continued to grow normally as shown by increase in body weight throughout the experiment, with no significant difference when compared to the controls. This relative safety of the herbal preparation was confirmed by the result of the cytotoxicity test, where concentrations as high as 1000 µg/mL of the tea extract didn't show any effect on the LLC-MK2 monkey kidney epithelial cell line.

Conclusion

The present work confirmed the hypogmyceamic effect of Afya tea in both non-diabetic and sptreptozotocin-induced diabetic wistar rats, thereby suggesting the antidiabetic potential of this herbal preparation. More interestingly, no sign of toxicity was noticed both *in vitro* on mammalian cell line and *in vivo* in mouse model, suggesting that the preparation could be safely used. Further studies including pharmacokinetics, evaluation of the stability pattern and mechanism of action, as well as clinical trials are warmly expected, to fully appreciate the suitability of this herbal preparation for treatment of diabetes mellitus in human.

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