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Magnesium Enhances the Antidiabetic Activity of *Lippia multiflora* Aqueous Extract on Glycemia, Lipid Profile and Cardiovascular Parameters in Streptozotocin-diabetic Rats

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Abstract: *Background & Aims:* According to studies, magnesium would have beneficial effects in the treatment of diabetes which prompted studies on the supplementation of medicinal substances with magnesium for the treatment of this disease. This work is situated in this context. It was conducted to investigate the effects of *Lippia multiflora* leaves aqueous extract supplemented with magnesium (LiMAE-Mg) on blood glucose, lipid profile and cardiovascular parameters in streptozotocin-diabetic rats and compared with diabetic and drug-treated rats. *Methods:* Diabetes was induced in adult male Wistar rats by intraperitoneal administration of streptozotocin (10 mg/kg). 7 groups of 5 STZ-diabetic rats were treated daily for 10 days with Glucophage (Glu 10 mg/kg), *Lippia multiflora* leaves aqueous extract (LiMAE 200, 400 and 600 mg/kg) and LiMAE-Mg (200, 400 and 600 mg/kg). One group of untreated diabetic rats (*u*DR) and one group of healthy rats (HeR) were the controls. After 10 days of treatment, some biological parameters were measured in 12 hr-fasted rats. *Results:* Diabetic rats had elevated levels of blood glucose (379.76%), glycosylated hemoglobin (HbA_{1c}), LDL-cholesterol, total cholesterol, triglycerides and cardiovascular parameters and decreased levels of blood insulin and HDL-cholesterol. Like Glu 10 mg/kg, LiMAE and LiMAE-Mg significantly ($p < 0.05$) reduced the disturbances caused by STZ in a dose-dependent manner. When diabetic rats were treated separately with LiMAE-Mg and LiMAE, a significant reduction in blood glucose (9.72 to 73.76%), HbA_{1c}, LDL-cholesterol, total cholesterol, triglycerides and cardiovascular parameters and an elevation in blood insulin and HDL-cholesterol were observed. However, the effects induced by LiMAE-Mg were greater at $p < 0.05$ than those of LiMAE, the extract of *Lippia multiflora* without magnesium. *Conclusion:* Supplementation with magnesium significantly increased the antidiabetic effects of *Lippia multiflora* leaves aqueous extract in streptozotocin-diabetic rats. However, future studies should be carried out on body weight, transaminases of regulatory organs, redox status and hematological parameters. Ultimately, these works will make it possible to better appreciate the beneficial effect of supplementation with magnesium of this medicinal plant extract in diabetic.

Keywords: *Lippia multiflora*, Diabetes, Magnesium, Streptozotocin, Antidiabetic Effect

1. Introduction

In the world and especially in Africa, more than 80% of the population uses plants for first care. They are for these populations an alternative to conventional medicine [1]. Plants, through their ability to produce secondary metabolites such as flavonoids, alkaloids, steroids, phenol compounds and other substances are used to restore health and cure many diseases including diabetes [2]. Diabetes mellitus or type 2 diabetes is a metabolic pathology characterized by endocrine dysfunction that requires lifelong treatment. Type 2 diabetes, representing more than 80% of diabetes cases, is marked by a disturbance in the homeostasis of lipids, proteins in addition to that of glucose [3].

In the body, most metabolic reactions depend on cofactors

supplied by food and drugs. And magnesium is one of the important cofactors. Magnesium (Mg^{2+}) is the most abundant intracellular divalent cation in cells, the second most abundant cellular ion next to potassium and the fourth cation overall in the human body [4]. Magnesium plays an important physiological role as a cofactor in more than 400 enzymatic reactions, including those of energy metabolism, such as carbohydrate oxidation and glucose transport mechanisms. It is also involved in the secretion, activity and insulin binding [5]. Diabetes is usually accompanied by hypomagnesemia which is a frequent morbid condition and especially with Type 2 diabetes [6]. This led many researchers in the early 1980s to suggest, on the one hand, the involvement of magnesium in insulin sensitivity and, on the other hand, a positive effect of magnesium supplementation in the treatment of Type 2 diabetes. Unfortunately, this beneficial effect of magnesium supplementation on type 2 diabetes has remained controversial [7]. Indeed, some studies have reported the beneficial effects of magnesium supplementation on metabolic control in patients with type 2 diabetes. Other studies, on the other hand, have shown no significant effect of magnesium supplementation on type 2 diabetes.

And it is in this controversial context that this study on *Lippia multiflora* leaves aqueous extract supplemented with magnesium was carried out in diabetic rats. This plant is widely used in traditional medicine to treat diabetes [8]. Allo [10] showed hypoglycemic and anti-hyperglycemic effects of the aqueous leaf extract of this medicinal plant in Wistar rats [9]. This plant would be non-toxic and tolerated by *Mus musculus* mices and *Rattus norvegicus* Wistar rats. Also, the aim of this work was to study the effect that magnesium supplementation could have on the antidiabetic action of *Lippia multiflora* leaves aqueous extract in streptozotocin-diabetic rats.

2. Materials and Methods

2.1. Plant Material and Extracts

Fresh leaves of *Lippia multiflora* (Verbenaceae) were harvested in February 2018 in Assouvoué, village of Toumodi Commune (Région du Bélier, Côte d'Ivoire). The fresh leaves were identified and authenticated by a Botany expert, Dr Rose-Monde Assi at the National Floristic Center (CNF) of Félix Houphouët-Boigny University (Abidjan, Côte d'Ivoire).

The plant aqueous extract was obtained according to the method used by Allo et *al.* [10]. The fresh leaves were washed and dried at room temperature (28 ± 2°C). Dried leaves were pulverized to powder with the use of a laboratory blender-About 100 g of powder were macerated during 24 hrs in distilled water (1L), thereafter filtered. An oven at a temperature of 50°C was used to concentrate the filtrate. And the concentrated extracts obtained (*Lippia multiflora* leaves aqueous extract: LiMAE) was stored at 4°C until experiments. LiMAE was supplemented with magnesium (1 g per 9 g LiMAE) to give the supplemented *Lippia multiflora* aqueous extract (LiMAE-Mg). The extracts were re-dissolved extemporaneously in normal saline (NaCl 0.9%) for the experiment [11, 12].

2.2. Animals and Ethics

Male Wistar rats (*Rattus norvegicus*) weighing 200-250 g and 8-12 weeks old were used for the tests. They were provided by the animal house of the Pharmaceutical and Biological Sciences UFR (Félix Houphouët-Boigny University). They were acclimatized for 2 weeks before being used for experiments. Animals were housed and maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light cycle (12/12 h). They were allowed free access to standard dry pellet diet and water *ad libitum*. Rats were treated according to good laboratory practices [13]. The experimental protocols were conducted in accordance with the protocols for the protection of experimental animals of the European Council on Legislation 2012/707 [14].

2.3. Chemicals Used

Streptozotocin (STZ 500 mg, Sigma-Adrich, USA), D(+) glucose monohydrate (Riedel-de Haën®, Germany), 0.1 M citrate buffer pH 4.5 (Merk®, USA), Nicotinamide (Sigma-Aldrich®, USA), Metformin hydrochloride (Glucophage® 10 mg, Sanofi-Aventis, France), Magnesium chloride crystals (ABCO®, Delbet, France) and Isoflurane (Forène®, Roche, France) were used. And Commercials Kit obtained from Spinreact (Spin).

2.4. Experimental Induction of Diabetes

Type 2 diabetes was induced following the modified protocol of Szkuelski [15]. After fasting overnight, the rats received an intraperitoneal injection of freshly prepared streptozocin (STZ) in citrate buffer solution (0.1 M, pH = 4.5). STZ was administered at 65 mg/kg. After 15 min, nicotinamide (230 mg/kg) has also been given to animals to stabilize STZ-induced lesions. Hyperglycemia was confirmed in rats by observation of clinical signs of polyuria, polydipsia, polyphagia and elevated blood glucose levels 72 h after treatment. Observation of these clinical signs continued for up to 21 days. Thus, from D14 to D21 after the treatment aimed at inducing type 2 diabetes, the rats which, in addition to the clinical signs mentioned above, presented a fasting blood glucose level (12-16 h) of 2-3 g/L associated with various other physical and metabolic disturbances have been diabetic [15-17].

2.5. Study Design

Four healthy rats (Group 1) and 32 STZ-diabetic rats divided into 8 groups of 4 animals (Groups 2-9) were used. Group 1 (HeR: healthy rats) and Group 2 (uDR: untreated-diabetic rats) received distilled water. Group 3 received Glucophage (Glu 10 mg/kg). Groups 4, 5, 6 were treated with LiMAE at 200, 400 and 600 mg/kg respectively while Groups 7, 8 and 9 received LiMAE-Mg at 200, 400 and 600 mg/kg respectively. Drugs were administered orally. And all animals had free access to water and normal diet during the experimentation. After 10 days of daily treatment, the 16

hr-fasted animals were used for the estimation of biological parameters [17].

2.6. Measurement of Biological Parameters

2.6.1. Glycemia

Blood was taken from the tail after a slight incision. Blood glucose was measured using reagent strips and a glucometer (Accu-Chek® Active, Germany).

2.6.2. Cardiovasular Parameters

Cardiovascular parameters were recorded with the Visitech BP 2000 device. Before each recording, the caudal vein was dilated by placing the rats in a heating stem for 5 min. Then, the rats were placed in the restraining cells and the pressure sensor was inserted on the tail of the animal. The systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (CF: cardiac frequency) values measured were displayed on the device's computer screen.

2.6.3. Biochemical Essays

Cardiovacular parameters and blood glucose measurements achieved, the 16 hr-fasted rats were immediately anaesthetized with Isoflurane (Forene®). A thoracotomy was performed and the blood samples were collected from the animals through cardiac puncture. Blood samples collected in non-heparinized tubes were allowed to clot for about 15 min and centrifuged at 3000 rpm for 5 min. Serum was separated from the clot with pasteur pipette and dispensed into clean tube for the measurement of the biochemical indices. Analysis of the selected serum biochemical indices were carried out on each sample. Parameters were measured using Chemistry Analyzer (HITACHI 704R® auto-analyser). Parameters studied were glycosylated hemoglobin (HBA_{1c}), blood insulin, total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C) and Triglycerides (TG).

2.7. Data Analysis

All the data were expressed as Mean ± Standard Error of Means (SEM). Statistical analyses were performed by one way analysis of variance (ANOVA) and differences between means were determined by Turkey's Multiple Comparison test using Graph Pad Prism 7.0 program (Microsoft, San Diego California, USA). A value of $p < 0.05$ was considered significant.

3. Results

3.1. Effects of Test Substances on Diabetes Biomarkers

3.1.1. Glycemia

Blood glucose of the diabetic rats (uDR) reached 3.19 ± 0.02 g/L against 0.84 ± 0.03 g/L in the healthy rats (HeR) an increase of 379.76% (Figure 1). Blood glucose values recorded with LiMAE (200 and 400 mg/kg) were 2.88 ± 0.07 g/L and 1.43 ± 0.11 g/L respectively. These blood glucose levels compared to those of untreated diabetic rats (uDR) correspond to respective decreases of 9.72% and 55.17%. Substantially equal values were obtained with LiMAE-Mg at the same doses ($p > 0.5$). When LiMAE was applied at a dose of 600 mg/kg, blood glucose was estimated at 0.95 ± 0.03 g/L, ie a decrease of 70.22% compared to untreated diabetic rats. With LiMAE-Mg 600 mg/kg, recorded glycemia was evaluated at 0.84 ± 0.02 g/L corresponding to a decrease of 73.67%. Like Glu 10 mg/kg, LiMAE-Mg 600 mg/kg restored blood glucose in STZ-diabetic rats. Indeed, this high dose of LiMAE-Mg induced in diabetic animals a glycemia which was statistically equal ($p > 0.5$) to that of non-diabetic rats (0.84 ± 0.03 g/L).

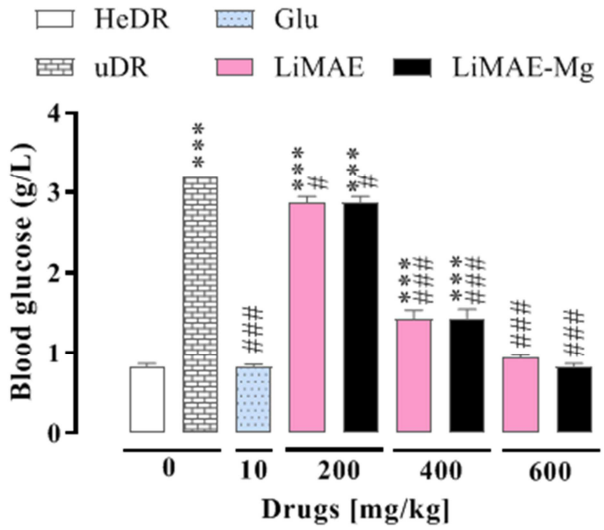

Figure 1. *Effects of drugs on blood glucose in STZ-diabetic rats. m ± sem, n = 4, *** p < 0,0001: significant difference with healthy rats (HeR). ### p < 0,0001, # p < 0,01: significant difference with untreated diabetic rats (uDR). STZ: streptozotocin; Glu: Glucophage; LiMAE: Lippia multiflora leaves aqueous extract; LiMAE-Mg: Lippia multiflora leaves aqueous extract supplemented with magnesium.*

3.1.2. Insulinemia

STZ caused a significant decrease ($p < 0.05$) in blood insulin level in rats. Insulinemia, which was 1.86 ± 0.01 µU/L (HeR), had fallen to 0.72 ± 0.03 µU/L (uDR), i.e. 61.29% reduction. Treatment of diabetic rats with different doses of test substances (LiMAE, LiMAE-Mg, Glu) led to a significant increase in insulinemia ($p < 0.05$) compared to untreated diabetic rats (Figure 2).

The blood insulin level of rats treated with LiMAE at a dose of 200 mg/kg was evaluated at 0.92 ± 0.03 µU/L giving an increase of 27.77% compared to untreated diabetic rats. At the same dose, LiMAE-Mg raised blood insulin level in treated rats to 1.17 ± 0.06 µU/L, an increase of 62.50% compared to untreated diabetic rats. At the high dose of 600 mg/kg, LiMAE and LiMAE-Mg induced a greater increase in blood insulin level. LiMAE caused an insulinemia of 1.23 ± 0.03 µU/L, i.e. an increase of 70.83% when LiMAE-Mg 600 mg/kg caused an insulinemia of 1.75 ± 0.04 µU/L, i.e. an increase 143.05% compared to untreated diabetic rats. Blood insulin was evaluated at 1.84 ± 0.01 µU/L in diabetic rats treated with Glu 10 mg/kg, i.e. 155.55% increase compared to untreated diabetic rats (uDR). Thus, at the high dose of 600 mg/kg, the blood insulin level induced by LiMAE-Mg was closer at $p > 0.05$ to that of the reference substance (Glu 10

mg/kg) in comparison to LiMAE, aqueous extract of *Lippia multiflora* not supplemented with magnesium.

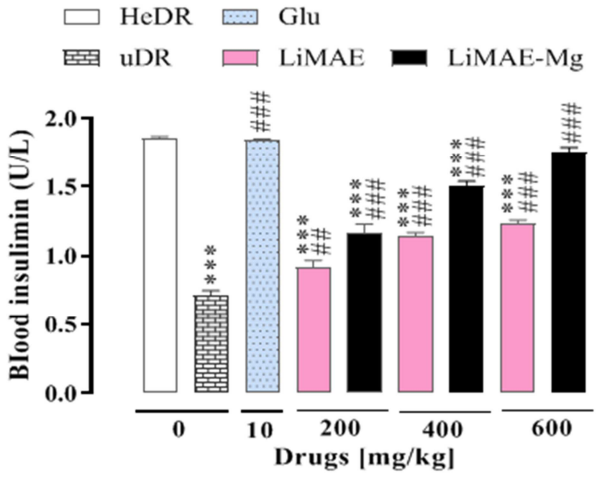

Figure 2. *Effects of drugs on blood insulin in STZ-diabetic rats. m ± sem, n = 4, *** p < 0,0001: significant difference with healthy rats (HeR). ### p < 0,0001, ## p < 0,02: significant difference with untreated diabetic rats (uDR). STZ: streptozotocin; Glu: Glucophage; LiMAE: Lippia multiflora leaves aqueous extract; LiMAE-Mg: Lippia multiflora leaves aqueous extract supplemented with magnesium.*

3.1.3. Glycosylated Hemoglobin

Figure 3 shows the effects of drugs on glycosylated hemoglobin. In untreated diabetic rats, a value of 71.96 ± 1.34% was recorded against 13.21 ± 1.56% in non-diabetic rats (HeR) (Figure 3).

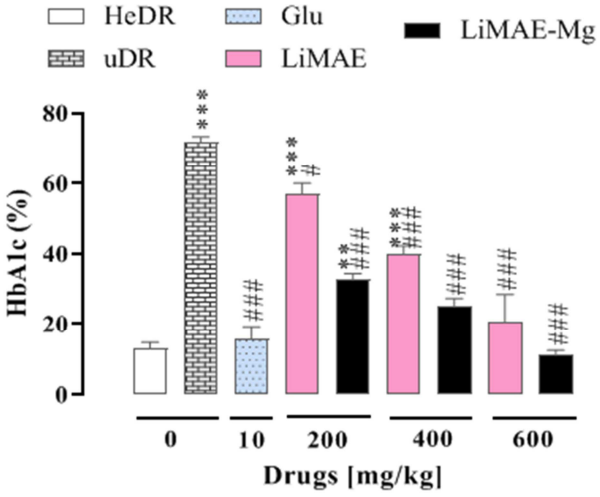

Figure 3. *Effects of drugs on glycosylated hemoglobin in STZ-diabetic rats. m ± sem, n = 4, *** p < 0,0001, ** p < 0,001: significant difference with healthy rats (HeR). ### p < 0,0001, # p < 0,05: significant difference with untreated diabetic rats (uDR). STZ: streptozotocin; HbA_{1c}: glycosylated hemoglobin, Glu: Glucophage; LiMAE: Lippia multiflora leaves aqueous extract; LiMAE-Mg: Lippia multiflora leaves aqueous extract supplemented with magnesium.*

Treatment of rats with the substances (LiMAE, LiMAE-Mg and Glu) significantly reduced ($p < 0.05$) the increase in HbA_{1c} levels observed in untreated diabetic rats. LiMAE at 200, 400 and 600 mg/kg increased HbA_{1c} from 71.96 ± 1.34% (uDR) to 57.03 ± 3.07%, 39.85 ± 2.43% and 20.86 ± 7.64% respectively in treated diabetic rats. In diabetic rats treated with LiMAE-Mg (200, 400 and 600 mg/kg), respective HbA_{1c} levels of 32.84 ± 1.57, 25.11 ± 2.28% and 11.44 ± 1.11% have been recorded. Finally, in the rats having received Gluc 10 mg/kg, an HbA1c level of 15.56 ± 3.70% was measured. LiMAE-Mg at 600 mg/kg caused a significant decrease of HbA_{1c} ($p < 0.05$) corresponding to a reduction of 84.20% compared to the value obtained in untreated diabetic rats against 71.01% for LiMAE 600 mg/kg and 78.38% for Glu 10 mg/kg.

3.2. Measurement of Biological Parameters

3.2.1. HDL-cholesterol

Diabetes caused a significant drop ($p < 0.05$) in HDL-C level to 0.12 ± 0.01 g/L against 0.52 ± 0.03 g/L in healthy rats (HeR), i.e. a reduction of 76.92%. Treatment of rats with *Lippia multiflora* aqueous extracts at 200 mg/kg increased HDL-C level. It evolved from 0.12 ± 0.02 g/L (uDR) to 0.18 ± 0.03 g/L (LiMAE) and 0.31 ± 0.02 g/L (LiMAE-Mg), i.e. respectively 50% and 158.33% increase. At a dose of 600 mg/kg, the extracts caused a very significant increase ($p < 0.05$) in the HDL-C level. This value increased to 0.51 ± 0.02 g/L (LiMAE) and 0.50 ± 0.01 g/L (LiMAE-Mg) compared to untreated diabetic rats giving respective increased rates of HDL -C of 325% and 316.67%. There was no significant difference at $p > 0.05$ between untreated diabetic rats compared to non-diabetic rats (HeR) which had an HDL-C level of 0.53 ± 0.04 g/L at the end of the experimental period. Glu 10 mg/kg increased HDL-C of 1100%. With Glu 10 mg/kg, the HDL-C level was evaluated at 1.44 ± 0.05 g/L (Figure 4).

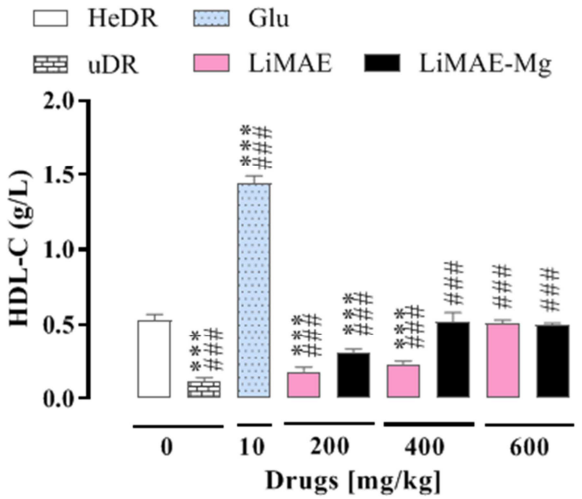

Figure 4. *Effects of drugs on HDL-cholesterol in STZ-diabetic rats. m ± sem, n = 4, *** p < 0,0001, ** p < 0,001: significant difference with healthy rats (HeR). ### p < 0,0001: significant difference with untreated diabetic rats (uDR); HDL-C: HDL-Cholesterol; STZ: streptozotocin; Glu: Glucophage; LiMAE: Lippia multiflora leaves aqueous extract; LiMAE-Mg: Lippia multiflora leaves aqueous extract supplemented with magnesium.*

3.2.2. LDL-cholesterol

Diabetes caused a significant increase of LDL-C level in rats, which rose from 0.16 ± 0.05 g/L (HeR) to 1.65 ± 0.03 g/L (uDR) corresponding to a increase of 931.25%. The treatment of diabetic rats with the LiMAE and LiMAE-Mg extracts at

doses of 200, 400 and 600 mg/kg allowed a progressive reduction of the LDL-C level. The most significant decrease was observed with the 600 mg/kg dose which reduced the LDL-C level from 1.56 ± 0.03 g/L (uDR) to 0.29 ± 0.02 g/L (LiMAE) and 0.19 ± 0.03 g/L (LiMAE-Mg). This corresponds to respective reduction percentages of 82.42% and 88%. In diabetic rats treated with Gluc 10 mg/kg, the LDL-C level was 0.27 ± 0.04 g/L and corresponded to a decrease of 83.84% compared to untreated diabetics (Figure 5).

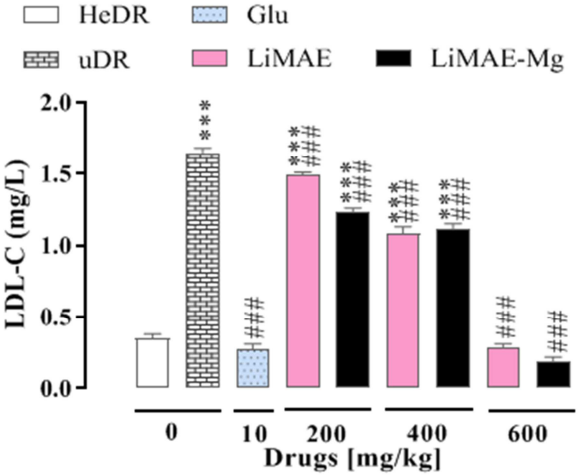

Figure 5. *Effects of drugs on LDL-cholesterol in STZ-diabetic rats. m ± sem, n = 4, *** p < 0,0001, ** p < 0,001: significant difference with healthy rats (HeR). ### p < 0,0001: significant difference with untreated diabetic rats (uDR). LDL-C: LDL-cholesterol; STZ: streptozotocin; Glu: Glucophage; LiMAE: Lippia multiflora leaves aqueous extract; LiMAE-Mg: Lippia multiflora leaves aqueous extract supplemented with magnesium.*

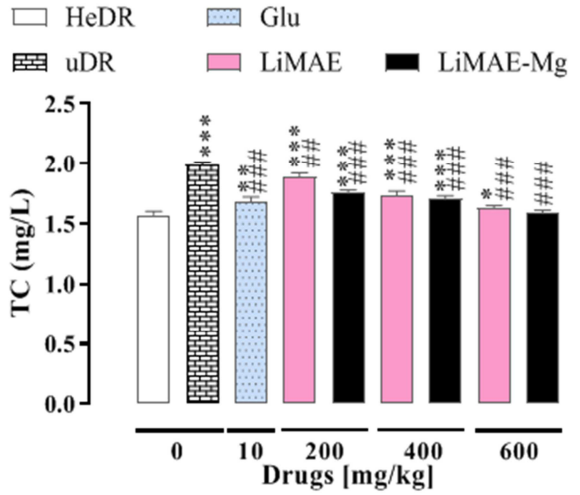

Figure 6. *Effects of drugs on Total cholesterol in STZ-diabetic rats. m ± sem, n = 4, *** p < 0,0001, ** p < 0,001: significant difference with healthy rats (HeR). ### p < 0,0001: significant difference with untreated diabetic rats (uDR). TC: Total cholesterol; STZ: streptozotocin; Glu: Glucophage; LiMAE: Lippia multiflora leaves aqueous extract; LiMAE-Mg: Lippia multiflora leaves aqueous extract supplemented with magnesium.*

3.2.3. Total Cholesterol

With diabetes, the TC level increased. It went from 1.57 ± 0.04 g/L (HeR) to 1.99 ± 0.02 g/L (uDR), i.e. a 27.75% increase. TC level decreased following treatment of diabetic rats with LiMAE and LiMAE-Mg (Figure 6).

The TC level fell from 1.99 ± 0.02 g/L (uDR) to 1.89 ± 0.03 g/L with LiMAE 200 mg/kg, i.e. a reduction of 5.02%. With LiMAE-Mg 200 mg/kg, the rate was estimated at 1.76 ± 0.02 g/L, i.e. 11.55% reduction. At 400 mg/kg, the results gave respective percentage reductions of 11.55% (LiMAE) and 14.07% (LiMAE-Mg). At 600 mg/kg, LiMAE and LiMAE-Mg significantly reduced the total cholesterol ($p < 0.05$) to 1.61 ± 0.02 g/L and 1.59 ± 0.02 g/L respectively giving reduction percentages of 18.09% and 20.10% in the same order. A decrease of 15.58% was obtained in diabetics who received Glu 10 mg/kg compared to untreated diabetics. With this substance, the TC level was evaluated at 1.68 ± 0.04 g/L.

3.2.4. Triglycerides

Like TC and LDL-C, TG level was increased at 119.67% in STZ-diabetic rats (1.34 ± 0.03 g/L) compared to healthy rats (0.61 ± 0.03g/L). The TG level had gone from 1.34 ± 0.03 g/L (uDR) to 1.24 ± 0.04 g/L (LiMAE 200 mg/kg) and 1.22 ± 0.04 g/L (LiMAE-Mg 200 mg/kg), i.e. respective reductions of 7.46% and 8.95%. At the 400 mg/kg dose, the TG level was estimated at 1.18 ± 0.06 g/L (LiMAE) and 1.14 ± 0.03 g/L (LiMAE-Mg) giving respective reductions of 11.94% and 14.92%. At a dose of 600 mg/kg, LiMAE and LiMAE-Mg significantly reduced at $p < 0.001$, the level of TG in treated rats compared to untreated diabetics. The respective decreases observed were 37.31% and 41.04%. Treatment of diabetic rats with Glu 10 mg/kg caused a drop in TG of 12.69%. Indeed, a TG value of 1.17 ± 0.04 g/L was recorded with Glu 10 mg/kg (Figure 7).

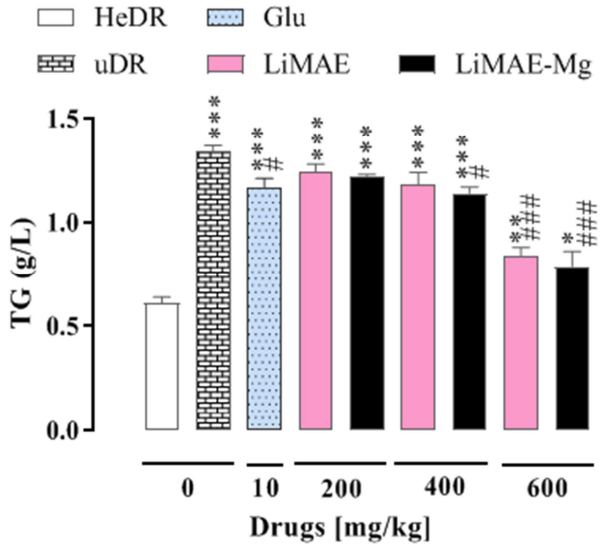

Figure 7. *Effects of drugs on triglycerides in STZ-diabetic rats. m ± sem, n = 4, *** p < 0,0001, ** p < 0,001: significant difference with healthy rats (HeR). ### p < 0,0001: significant difference with untreated diabetic rats (uDR). TG: Triglycerides; STZ: streptozotocin; Glu: Glucophage; LiMAE: Lippia multiflora leaves aqueous extract; LiMAE-Mg: Lippia multiflora leaves aqueous extract supplemented with magnesium.*

3.3. Effects of Test Substances on Cardiovascular Parameters

Diabetes caused a significant increase of the arterial blood

pressure in rats illustrated by cardiovascular parameters (SBP, DBP and CF) which were strongly modified (Figure 8). The values of these parameters increased in diabetic rats.

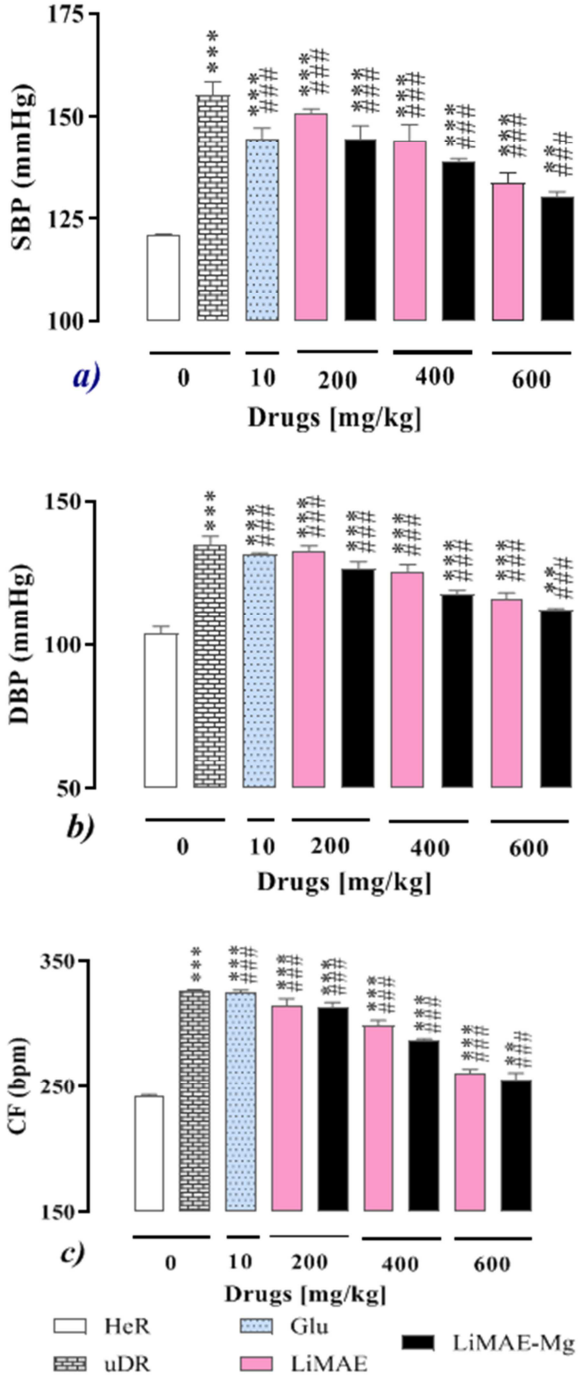

Figure 8. *Effects of drugs on the cardiovascular parameters in STZ-diabetic rats. a) Systolic blood pressure (SBP), b) Diastolic blood pressure (DBP), c) Cardiac frequency (CF); m ± sem; n = 4; *** p < 0,0001, ** p < 0,001: significant difference with healthy rats (HeR); ### p < 0,0001: significant difference with untreated diabetic rats (uDR); STZ: streptozotocin; Glu: Glucophage, LiMAE: Lippia multiflora leaves aqueous extract, LiMAE-Mg: Lippia multiflora leaves aqueous extract supplemented with magnesium.*

In contrast, treatment of animals with LiMAE, LiMAE-Mg and Glu separately corrected this elevation of cardiovascular parameters in diabetic rats. Indeed, diabetes caused an elevation of SBP, DBP and CF in rats. SBP, DBP and CF increased from 121 ± 0.07 mmHg, 103.94 ± 2.28 mmHg and 242 ± 1.52 bpm respectively in healthy rats to 155.21 ± 3.27 mmHg, 135.00 ± 2.7828 mmHg and at 326 ± 10.41 bpm in untreated diabetic rats. With the induction of diabetes, increases of 28.05% (SBP), 31.06% (DBP) and 34.71% (CF) were recorded.

Treatments of diabetic rats with LiMAE, LiMAE-Mg led to a significant decrease at $p < 0.05$ of cardiovascular parameters with doses of 400 and 600 mg/kg of these cardiovascular parameters. At a dose of 200 mg/kg of LiMAE, 150.71 ± 1.20 mmHg (SBP), 132.88 ± 1.59 mmHg (DBP) and 315 ± 5.5 bpm (CF) were measured, giving respectively reductions of 2.89%, 1.57% and 3.37% compared to untreated diabetic rats. It was recorded 144.11 ± 3.41 mmHg (SBP), 126.71 ± 2.28 mmHg (DBP) and 313 ± 4.35 bpm (CF) in diabetic rats which received LiMAE-Mg 200 mg/kg. These values correspond to respective reductions of 7.02%, 6.14% and 3.98%. At the dose of 400 mg/kg, LiMAE-Mg induced a more pronounced decrease in SBP, DBP and CF than LiMAE. Values of 138.76 ± 0.86 mmHg (SBP), 117.73 ± 1.30 mmHg (DBP) and 287 ± 1.30 bpm (CF) i.e. respective decreases of 10.51%, 12.79% and 11.96% respectively against 7.16% (SBP), 6.87% (DBP) and 8.28% (CF) for LiMAE were measured. At a dose of 600 mg/kg, LiMAE and LiMAE-Mg caused a more significant decrease at $p < 0.05$ of SBP, DBP and CF. The measured values were respectively 133.87 ± 2.35 mmHg, 115.81 ± 2.29 mmHg and 261 ± 3.21 bpm corresponding in the same order to reductions of 13.74%, 14.21% and 19.93% for LiMAE. Regarding LiMAE-Mg, 130.35 ± 1.24 mmHg (SBP), 112.10 ± 0.56 mmHg (DBP) and 256 ± 4.93 bpm (CF) were recorded, i.e. respective reductions of 16.01%; 16.96% and 21.93%. Glu 10 mg/kg did not significantly reduce SBP, DBP and CF at $p < 0.05$.

4. Discussion

4.1. Effect on Blood Glucose

Results obtained during the study showed a modification of biological parameters by diabetes. Indeed, there was an increase in glycemia and glycosylated hemoglobin associated with an insulinemia decrease in diabetic rats. The treatments of diabetic animals with plant extracts (LiMAE, LiMAE-Mg) and Glucophage led to a normalization of these parameters.

Diabetes mellitus is a collection of metabolic disorders characterized by hyperglycemia [18, 19]. These metabolic disorders include alterations in carbohydrate, fat and protein metabolism associated with absolute or relative deficiencies in insulin secretion and/or insulin action [19]. The induction of experimental diabetes mellitus in animal models is essential for knowledge and understanding of various aspects of pathogenesis [20]. This ultimately aims to develop new therapies. Currently, the two most widely used chemicals to induce experimental diabetes are alloxan and streptozocin (STZ). According to Pinheiro et *al.* [21], STZ and alloxan would have been used at 69% and 31% respectively in animal models of chemically induced diabetes during the last decades.

Alloxan and STZ are toxic glucose analogues that preferentially accumulate in pancreatic β-cells through the glucose transporter GLUT2. Their diabetogenic actions are due to their ability to destroy β-pancreatic cells. The main cause of STZ-induced β-cell death is DNA alkylation by the nitrosourea moiety of this substance. However, NO production and reactive oxygen species may also be involved in DNA fragmentation and other deleterious effects of STZ [22].

Insulin-glucagon balance to maintain stable glycemia, called glucose homeostasis, is crucial for the utilization of glucose by the liver, muscle, and adipose tissue [23]. In this study, STZ was used to induce hyperglycemia in rats because it is a DNA methylating agent and acts by damaging β-pancreatic cells [24]. One of the main features of diabetes mellitus is excessive glucose concentration (hyperglycemia) caused by insulin deficiency. The treatment of diabetic rats with LiMAE, LiMAE-Mg and Glucophage caused a significant decrease in glycemia (blood glucose level) and glycosylated hemoglobin as well as an increase in insulinaemia. The glycemia decrease observed is consistent with the results of Fah et *al.* [8] on the use of *Lippia multiflora* leaves to treat pregnant women suffering from gestational diabetes in rural Benin. The improvement of these symptoms that characterize type 2 diabetes were also observed by some authors [25, 26]. These effects could be explained by the composition of these extracts in bioactive phytochemical molecules especially in flavonoids. Indeed, previous works have highlighted the presence of polyphenols, flavonoids, saponins, alkaloids, tannins, sterols and terpenes in the aqueous and ethanolic extracts of *Lippia multiflora* leaves [10, 27]. These metabolites have the ability to regulate blood glucose through several signaling pathways. Saponins, polyphenols and in particular flavonoids would exert a hypoglycemic activity by inhibiting the intestinal absorption of glucose and glycogenolysis. The Inhibition of the activity of several enzymes such as α-amylase, α-glucosidase, glucose-6-phosphatase (G6Pase) would lead to a reduction in the blood bioavailability of glucose [28, 29]. After administration of extracts (LiMAE and LiMAE-Mg) rich in polyphenols and polyterpenes to diabetic rats, insulin concentration increased significantly especially with LiMAE-Mg. This result is in agreement with those of previous works. These showed that the terpene-rich leaves would be involved in the stimulation of β-pancreatic cells and insulin secretion [30, 31].

HbA_{1c} is a major tool for assessing blood glucose control and it has strong predictive value for diabetes and its complications. Induction of diabetes by STZ increased the percentage of HbA_{1c} in rats.

4.2. Effects on Cardiovascular Parameters

One of the consequences of the induction of diabetes in rats by STZ was the disruption of cardiovascular parameters such as SBP, DBP and heart rate (HR) in diseased animals. Arterial hypertension is a permanent elevation in blood pressure (BP) characterized by an increase in cardiovascular parameters [32, 33]. However, the treatment of diabetic rats with LiMAE and LiMAE-Mg induced a significant decrease in these parameters. These results are in agreement with those of Etou-Ossibi *et al.* [34] who showed that *Lippia multiflora* aqueous extract prevents and treats DOCA- NaCl-induced arterial hypertension in rats. It is, moreover, this hypotensive effect of *Lippia multiflora* aqueous extract which would justify the use of this plant in the treatment of arterial hypertension in traditional medicine [35]. The decrease in cardiovascular parameters in diabetic rats was greater with the administration of LiMAE-Mg compared to LiMAE.

4.3. Effects on Lipid Profile

A marked increase in lipid parameters TG, TC, LDL-C and a decrease in the level of HDL-C were observed in diabetic rats after induction of diabetes with STZ. During the diabetic state, insulin deficiency contributes to various metabolic disorders and self-regulatory mechanisms in the body. In the normal state, insulin activates the action of lipolytic hormones on peripheral fat deposits which hydrolyzes triglycerides and prevents the mobilization of fatty acids [36].

However, insulin deficiency inactivates lipoprotein lipase which promotes the hepatic conversion of free fatty acids into phospholipids and cholesterol and finally released into the blood, resulting in elevated serum phospholipid levels [37]. The significant decrease in TG, TC, LDL-C levels and significant increase in HDL-C level by LiMAE and LiMAE-Mg in diabetic rats imply that these extracts might possess insulin-like activity which would be useful in reducing the incidence of lipid complications. This activity of *Lippia mulflora* extracts could be due to the phenolic compounds they contain as reported by Gupta et *al.* [38]. According to these authors, phenolic compounds are active biological metabolites of most medicinal plants that have hypolipidemic and antidiabetic properties.

4.4. Some Mechanisms Underlying the Potentiating Action of Magnesium

Magnesium (Mg^{2+}) is the most abundant bivalent intracellular cation in cells, the second most abundant cellular ion next to potassium and the fourth most abundant cation overall in the human body [4]. Magnesium plays an important physiological role due to its role as a cofactor in over 400 enzymatic reactions, including those involved in energy metabolism. It is involved in the oxidation of carbohydrates, the mechanisms of glucose transport, as well as the secretion, activity and binding of insulin [5]. Diabetes is usually accompanied by hypomagnesemia which is a common disease condition especially with type 2 diabetes [6].

The importance of magnesium on insulin sensitivity was suggested in the early 1980s and led to clinical evidence. Some studies have reported beneficial effects of magnesium supplementation on metabolic control in individuals with type 2 diabetes. On the other hand, other studies have shown no significant effect of magnesium supplementation on type 2 diabetes. As a result, the effects of magnesium supplementation have remained controversial in the literature

[7]. In the present study, the aqueous extract of *Lippia multiflora* was supplemented with magnesium for the treatment of type 2 diabetic rats. The results showed that the rats treated with the supplemented aqueous extract (LiMAE-Mg) had a significant decrease at $p < 0.05$ in the glycemia and the percentage of HbA_{1c} and consequently an increase in the insulinaemia of the diabetic rats.

The values obtained with LiMAE-Mg are very close to or even better than those of Glucophage, the reference antidiabetic substance, and those of normoglycemic control rats. These results support those of Fah et *al.* [8] on the use of Lippia multiflora leaves in the treatment of gestational diabetes. The rise in insulin levels and the decrease in glycemic levels observed in diabetic rats treated with LiMAE-Mg at different doses and especially at the dose of 600 mg/kg bw agree with the results of previous studies [39, 40]. These authors investigated the role of magnesium supplementation of Metformin on β-cell regeneration and insulin sensitivity by scanning the important gene PDX-1. The PDX-1 is an important protein and transcription factor essential for the development of the pancreas. Indeed, the synthesis and secretion of insulin are stimulated by an external factor, glucose, and by an internal factor, the protein coded by the PDX-1 gene. Also, its upregulation may signal regeneration of β cells in the pancreas responsible for insulin secretion. Supplementation of *Lippia multiflora* with magnesium (LiMAE-Mg) showed better glycemic regulation compared to the non-supplemented aqueous extract (LiMAE). This up-regulation suggests that pancreatic β-cells have undergone a development that could be a regeneration of the population of these cells in rats poisoned by STZ. This would have led to increased insulin secretion in rats treated with LiMAE-Mg. This result could be based on the previous works [41, 42] on the regeneration of pancreatic β cells. As well as glycemia, many other factors related to the complication of diabetes showed improvement in diabetic rats treated with *Lippia multiflora* aqueous extract supplemented with magnesium (LiMAE-Mg). Thus, cholesterol levels and cardiovascular parameters (DBP, SBP and CF) in diabetic rats experienced a normalization compared to those of non-diabetic rats. Magnesium deficiency is one of the main factors in cardiovascular diseases such as arrhythmias, arterial calcifications, atherosclerosis, heart failure, hypertension and thrombosis [43]. In other words, disorders of magnesium metabolism are one of the main causes of cardiovascular disease. Hypertensive patients on long-term treatment with thiazide diuretics should therefore be monitored for magnesium deficiency, especially those with additive risk factors, such as age (over 60 years), insulin resistance, cardiovascular diseases (hypertension, arrhythmias), insufficient dietary intake and renal dysfunction [44]. Consistent oral supplementation with magnesium is associated with better blood pressure control, improved endothelial function, and improved atherosclerosis in treated hypertensive and diabetic patients [43].

5. Conclusion

Lippia multiflora leaves aqueous extract reduced the physical and metabolic disturbances caused by type 2 diabetes in Wistar rats. This beneficial action of *Lippia multiflora* extract on diabetes was enhanced by magnesium as illustrated by the results of this study. Compared to *Lippia multiflora* aqueous extract (LiMAE), the extract supplemented with magnesium (LiMAE-Mg) allowed a greater reduction in disturbances of markers of blood glucose, lipid profile and cardiovascular parameters in diabetic rats.

Lippia multiflora leaves aqueous extract supplemented with magnesium could be an attractive way to prevent and treat diabetes and its complications. For this, additional studies must be carried out on the regulatory organs and various other biomarkers affected by diabetes. Indeed, persistent hyperglycemia linked to diabetes can, over time, affect all organs and in particular the brain, eyes, kidneys and liver.

Abbreviations

CF: Cardiac frequency
CNF: National Floristic Center
DBP: Distolic bood pressure
HbA_{1c}: Glycosylated hemoglobin
HDL-C: HDL-cholesterol
HeR: Haelthy rats
LDL-C: LDL-cholesterol
LiMAE: *Lippia multiflora* aqueous leaf extract
LiMAE-Mg: LiMAE supplemented with magnesium
NaCl: Sodium chloride
SBP: Systolic blood pressure
STZ: Streptozotocin
TC: Total cholesterol
TG: Triglycerides
*u*DR: Untreated diabetic rats

Availability of Data and Materials

The datasets used and/or analyzed in the current study are available from the corresponding author on reasonable request.

Authors' Contribution

Jacques Yao Datte proposed the research idea. Yapo Fulgence Allo collected data. Ziehi Fidele Kpahe carried out statistics analysis and prepared the first draft. Brou Andre Konan supervised the work, revised the manuscript for scientific content and did the language check. All authors read and approved the final manuscript.

Competing Interests

The authors declare that there is no conflict of interest.

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References

[1] Ambe, G. A. (2001). Edible wild fruits in *Guinean savannas* of the Ivory Coast: State of the knowledge by local population, the Malinké. Biotechnology, Agronomy, Society and Environment, 5 (1), 43-58.

[2] Djoupo, A., Yapi, H. F., Dasse, S. R., Djyh, G. B., N'guessan, J. D., & Djaman, A. J. (2015). Positive effects of aqueous and ethanolic extracts of stem bark from *Trichilia emetica* (Meliaceae) on cellular immunity markers in rats. IJBCRR, 8 (3), 1-6. https://doi.org/10.9734/IJBCRR/2015/19629

[3] Simoni, Y. (2013). Innate immunity in diabetes mellitus. Human medicine and Pathology. René Descartes University - Paris V, France. https://tel.archives-ouvertes.fr/tel-00944309

[4] Barbagallo, M., & Dominguez, L. J. (2007). Magnesium metabolism in type 2 diabetes mellitus, metabolic syndrome and insulin resistance. Archives of Biochemistry and Biophysics, 458, 40-47. https://doi.org/10.1016/j.abb.2006.05.007

[5] Hruby, A., O'Donnell, C. J., Paul, F. J., Meigs, J. B., Udo, H., & McKeown, N. M., 2014. Magnesium intake is inversely associated with coronary artery calcification: The Framingham Heart Study**.** JACC Cardiovasc. Imaging, 7, 59-69. https://doi.org/10.1016/j.jcmg.2013.10.006

[6] IDF (International Diabetes Federation), 2016. Talking the costs of diabetes. Diabetes Voice, 61, 1-40.

[7] Fapohunda, O. (2018)**.** Synergistic insulinotropic effect of metformin–Mg^{2+} adjunct supplement: A case study of streptozotocin induced type 2 diabetes in Sprague Dawley rats. Journal of Diabetes Metabolic Disorder and Control, 5, 38-46. https://doi.org/10.15406/jdmdc.2018.05.00136

[8] Fah, L., Klotoé, J. R., Dougnon, V., Koudokpon, H., Fanou, V. B. A., Dandjesso, C., et al. (2013)**.** An ethnobotanical study of plants used in the treatment of diabetes in pregnant women in Cotonou and Abomey-Calavi (Benin). Journal of Animal & Plant Sciences, 18, 2647-2658.

[9] Allo, F. Y. (2020). Antidiabetic activity of aqueous extract of *Lippia multiflora* (Verbenaceae) harvested in Toumodi (Bélier Region) supplemented with magnesium in type 2 diabetic rats. PhD, Felix Houphouet-Boigny University, Abidjan, Ivory Coast, 150 p.

[10] Allo, F. Y., Konan, A. B., Méité, S., & Datté, J. Y. (2020). Acute and sub-acute toxicity studies of the aqueous leaf extract of *Lippia multiflora* from the Bélier Region (Côte d'Ivoire). Asian Journal of Emerging Research, 2 (1), 43-53. https://doi.org/10.3923/AJERPK.2020.43.53

[11] Koko, B. K., Konan, A. B., Kouacou, F. K. A., Djetouan, J. M. K., & Amonkan, A. K. (2019). Galactagogue effect of *Euphorbia hirta* (Euphorbiaceae) aqueous leaf extract on milk production in female Wistar rats. Journal of Biosciences and Medicines, 7, 51-65. https://doi.org/10.4236/jbm.2019.79006

[12] Konan, B. A., Kpahé, Z. F., Koko, K. B., & Adepo, Y. P. (2022). Diuretic activities of root bark aqueous and ethanolic extracts of *Parquetina nigrescens*. I-Effects on urinary excretion in Wistar rat. Journal of Drug Delivery and Therapeutics, 12, 57-61. http://dx.doi.org/10.22270/jddt.v12i3.5318

[13] OCDE (1998). OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring. ENV/MC/CHEM, 98, 17. https://doi.org/10.1787/20777868

[14] European Union (2012). Commission implementing decision of 14 november 2012 establishing a common format the submission of the information pursuant to Directive 2010/63/EU of the Europeen parliament and the council on the protection of animals used for scientific purposes (notified under document C (2012) 8064) text with EEA relevance. Special Education Croatian, 15 (28), 163-180. http://data.europa.eu/eli/dec_impl/2012/707/oj

[15] Szkudelski, T. 2001. The mechanism of alloxan and streptozotocin action β cells of the rat pancreas. Physiology Research, 50, 536-546. PMID: 11829314.

[16] Ramesh, B., Saravanan, R., & Pugalendi, K. V. (2006). Effect of dietary substitution of groundnut oil on blood glucose, lipid profile, and redox status in streptozotocin-diabetic rats. Yale Journal of Biology and Medicine, 79, 9-17. https://pubmed.ncbi.nlm.nih.gov/17876371

[17] Samir, D., Iman, S., Maroua, Z., & Zineb, M. (2020). Anti-diabetic and anti-oxidative activities of Algerian *Oudneya africana* extract in alloxan induced diabetic rats. Asian Journal of Emerging Research, 2, 137-145.

[18] Navarro-Gonzalez, J. F., & Mora-Fernández, C. (2008). The role of inflammatory cytokines in diabetic nephropathy. Journal of American Society of Nephrology, 19, 433-442. https://doi.org/10.1681/asn.2007091048

[19] Daisy, P., & Kani, F. G. J. (2013). Hypolipidemic and hepatoprotrctive effects of *Cassia auriculata* Linn back extracts on streptozotocin induced diabetics in male Wistar albinos rats. Asian Journal of Pharmaceutical Clinical Research, 6 (2), 43-48.

[20] Abeeleh, M. A., Ismail, Z. B., Alzaben, K. R., Sami, A-H., Al-Essa., M., Abeeleh, J. A. et al. 2009. Induction of diabetes mellitus in rats using intraperitoneal streptozotocin: A comparison between 2 strains of rats. European Journal of Scientific Research, 32 (3), 398-402.

[21] Pinheiro, L. S., de Melo, A. D., Andreazzi, A. E., Caires-Júnior, L. C., Costa, M. B., & Garcia, R. M. G. (2011). Protocol of insulin therapy for streptozotocin-diabetic rats based on a study of food ingestion and glycemic variation. Scandinavian Journal of Laboratory Animal Science, 38 (2), 117-127.

[22] Hajzadeh, M. A. R., Rajaei, Z., Ghamami, G., & Tamiz, A., 2011. The effect of *Salvia officinalis* leaf extract on blood glucose in streptozotocin-diabetic rats. Pharmacologyonline, 1, 213-220.

[23] Ajiboye, B. O., Shonibare, M. T., & Oyinloye B. E., 2020. Antidiabetic activity of watermelon (Citrullus lanatus) juice in alloxan-induced diabetic rats. Journal of Diabetes & Metabolic Disorders, 19 (1), 343-352. https://doi.org/10.1007%2Fs40200-020-00515-2

[24] Ahmed, D., Kumar, V., Verma, A., Shukla, G. S., & Sharma, M., 2015. Antidiabetic, antioxidant, antihyperlipidemic effect of extract of *Euryale ferox* Salisb. with enhanced histopathology of pancreas, liver and kidney in streptozotocin induced diabetic rats. Springerplus, 4, 315. https://doi.org/10.1186/s40064-015-1059-7

[25] Eidi, M., Eidi, A., & Zamanizadeh, H., 2005. Effect of *Salvia officinalis* L. leaves on serum glucose and insulin in healthy and streptozotocin-induced diabetic rats. Journal of Ethnopharmacology, 100, 310-313. https://doi.org/10.1016/j.jep.2005.03.008

[26] Lima, C. R., Vasconcelos, C. F. B., Costa-Silva, J. H., Maranhão, C. A., Costa, J., Batista, T. M. et al., 2012. Anti-diabetic activity of extract from *Persea americana* Mill. leaf via the activation of protein kinase B (PKB/Akt) in streptozotocin-induced diabetic rats. Journal of Ethnopharmacology, 141 (1), 517-525. https://doi.org/10.1016/j.jep.2012.03.026

[27] Masengo, C. A., Bongo, G. N., Robijoana, B., Ilumbe, G. B., Ngbolua Koto-Te-Nyiwa, J.-P., & Mpiana, P. T., 2020. Quantitative ethno-botanical study and sociocultural value of *Lippia multiflora* Moldenke (Verbenaceae) in Kinshasa city, Democratic Republic of the Congo. Revue Marocaine des Sciences Agronomiques et Vétérinaires, 9 (1), 93-101.

[28] Jung, M., Park, M., Lee, H. C., Kang, Y.-C., Kang, E. S., & Kim S. K., 2006. Antidiabetic agents from medicinal plants. Current Medicinal Chemistry, 13 (10), 1203-1218. https://doi.org/10.2174/092986706776360860

[29] Kim, K., Choi, J. S., Choi, E, Nieman, C. L., Jin, H. J., Lin, F. R. et al. (2016). Effects of community-based health worker interventions to improve chronic disease management and care among vulnerable populations: A systematic review. American Journal Public Health, 106 (4), e3-e28. https://doi.org/10.2105/ajph.2015.302987

[30] Bhattacharya, A., Tiwari, P., Sahu, P. K., & Kumar, S. (2018). A review of the phytochemical and pharmacological characteristics of Moringa oleifera*. Journal of Pharmacy and Bioallied Science, 10, 181-191.* https://doi.org/10.4103%2FJPBS.JPBS_126_18

[31] Konmy, B. B. S., Olounladé, P. A., Allou, S.-Y. D., Azando, E. V., & Hounzangbé-Adoté, M. S. (2016). A review on phytochemistry and pharmacology of *Moringa oleifera* (Moringaceae). Journal of Pharmacognosy and Phytochemistry, 5 (5), 325-330.

[32] Wolf-Maier, K., Cooper, R. S., Banegas, J. R., Giampaoli, S., Hense, H.-W., Joffres, M. et al. (2003). Hypertension prevalence and blood pressure levels in 6 European countries, Canada and the United States. JAMA, 289 (3), 2363-2369. https://doi.org/10.1001/jama.289.18.2363

[33] Boga, G. L. (2015). Evaluation of the antihypertensive effects of the total dichloromethane-ethanol extract of *Moringa morindoides* (Baker) Milne-Redh (Rubiaceae) (ETDE) leaves on induced arterial hypertension in rats. PhD. Felix Houphouet-Boigny University, Abidjan, Ivory Coast, 111 p.

[34] Etou-Ossibi, A. W., Dimo, T., Elion-Itou, R. D. G., Nsondé-Ntandou, G. F., Nzonzi, J., Bilanda, D. C. et al. (2012). Effects of aqueous extract of *Lippia multiflora* Moldenke on the arterial hypertension induced by DOCA-salt in the rat. Phytothérapie, 10 (6), 363-368. https://doi.org/10.1007/s10298-012-0748-4

[35] Etou-Ossibi, A. W., Elion-Itou, R. D. G., Nzonzi, J., Nsonde-Ntandou, G. F., Dimo, T., Ouamba, J. M. et al. (2014). Effects of aqueous extract of *Lippia multiflora* Moldenke (Verbenaceae) on the blood pressure, the heart rate and on electrocardiogram waves at the normotensive rat. Revue CAMES – Série Pharmacopée et Médecine Traditionnelle Africaine, 17 (1), 1-9.

[36] Briones, E. R., Mao, S. J. T., Palumbo, P. J., O'Fallon, W. M., Chenoweth, W., & Kottke, B. A. (1984)**.** Analysis of plasma lipids and apolipoproteins in insulin-dependent and noninsulin-dependent diabetics. Metabolism, 33, 42-49. https://doi.org/10.1016/0026-0495(84)90160-4

[37] Pushparaj, P. N., Low, H. K., Manikandan, J., Tan, B. K. H., & Tan, C. H. (2007). Anti-diabetic effects of *Cichorium intybus* in streptozotocin-induced diabetic rats. Journal of Ethnopharmacology, 111, 430-434. https://doi.org/10.1016/j.jep.2006.11.028

[38] Gupta, R. K., Dharmendra, K., Chaudhary, A. K., Maithani, M., & Singh, R. (2012). Antidiabetic activity of *Passiflora incarnata* Linn in streptozotocin-induced diabetes in mice. Journal of Ethnopharmacology, 139, 801-806. https://doi.org/10.1016/j.jep.2011.12.021

[39] Melloul, D., Marshak, S., & Cerasi, E. (2002). Regulation of pdx-1 gene expression. Diabetes, 51 (3), 320-325. https://doi.org/10.2337/diabetes.51.2007.s320

[40] Fapohunda, O., Ogunleye, F. A., Ogunwa, T. H., Omotuy, I. O., Samuel, T. T., Inyang, K. O., et al., 2021. Metformin-Mg2+ adjunct therapy synergistically modulates insulin and PDX-1 gene signatures in STZ-NAD induced diabetic model. Endocrinology & Metabolism International Journal, 8 (3): 66-71. https://doi.org/10.15406/emij.2020.08.00281

[41] Butler, P. C., Meier, J. J., Butler, A. E., & Bhushan, A. (2007)**.** The replication of beta cells in normal physiology, in disease and for therapy. Nature Clinical Practice Endocrinology & Metabolism, 3 (11), 758-768. https://doi.org/10.1038/ncpendmet0647

[42] Emaleku, S. A. (2019). Hypoglycemic effect of Asheitu adams bitter in diabetic experimental animals. Acta Scientific Medical Sciences, 3 (2), 70-76.

[43] Gröber, U., Schmidt, J., & Kisters, K. (2015). Magnesium in prevention and therapy. Nutrients, 7, 8199-8226. https://doi.org/10.3390%2Fnu7095388

[44] Cunha, A. R., D'El-Rei, J., Medeiros, F., Umbelino, B., Oigman, W., Touyz R. M. et al. 2017. Oral magnesium supplementation improves endothelial function and attenuates subclinical atherosclerosis in thiazide-treated hypertensive women. Journal of Hypertension, 35, 89-97. https://doi.org/10.1097/hjh.0000000000001129