Original Research Article

Effects of *Erythrina senegalensis* (Fabaceae) stem bark aqueous extract in cardiovascular complications on hypertensive diabetic rat

Abstract

***Background and aim*:** Cardiovascular complications are the main cause of morbidity and mortality in hypertensive diabetic patients. They develop due to microvasculopathy, myocardial hypertrophy and cardiac fibrosis. In the present study, the activity of aqueous extract of *Erythrina senegalensis* on cardiovascular complications in diabetic hypertensive rats was evaluated.

Experimental procedure: The model of hypertensive diabetic rats was obtained by daily oral administration of sucrose (15%) and 40° ethanol at doses of 1.5 and 5 g/kg respectively for six weeks, followed by an intravenous injection of streptozotocin (35 mg/kg). After four weeks of complications, the diabetic hypertensive rats were divided into six groups of 5 animals each, and received the different treatments for four weeks where blood glucose level was taken weekly. At the end of the treatments, the animals were anaesthetised and the arterial pressures and electrocardiograph were recorded by the direct method. Then the aorta and heart were removed for biochemical by colorimetric method and histological analysis.

***Results and conclusion*:** In the diabetic hypertensive animals the results showed chronic hyperglycaemia associated with high blood pressure. On the electrocardiograph there was a cardiac arrhythmia manifested by a significant increase in the duration of the PR, QRS, RR, QTc segments, ST interval, P, QRS and T waves, a decrease in the amplitude of the QRS and T waves with an increase in P. There was a significant increase in cholesterol, triglyceride atherogenic index and a decrease in HDL-cholesterol with a change in the cardiac ionogram and oxidative status in the aorta and heart. The different treatments improved these parameters and cardiovascular function. These results suggest that the extract of *E. senegalensis* by the presence of several bioactive compounds would possess cardioprotective, hypoglycaemic, hypotensive and antioxidant properties.

Key words: hypertension, diabetes, electrocardiograph, cardiovascular complications, *Erythrina senegalensis*, rats

1 Introduction

The cardiovascular system (CVS) consists of the heart and a network of vessels through which blood or lymph circulates. The blood circulation is composed of two networks: the arterial network where blood flows from the heart to the tissues and the venous network where blood flows from the tissues to the heart [1]. The heart is a hollow muscle with a rhythmic contraction whose function is to ensure the progression of blood inside the vessels [1]. The cardiac conduction system includes the sinoatrial node (SAN), atrioventricular junction (AVJ), bundle branches (BB) and Purkinje fibres (PF) [2]. This network of specialised myocytes has unique molecular, anatomical and functional properties, enabling it to maintain the electrical activity of the heart [3]. In clinical pathology, obesity, atherosclerosis, hypertension, diabetes mellitus and associated complications have common pathological links with the metabolic syndrome [4]. These diseases have become a major health threat in the modern world, affecting millions of people and causing an economic downturn [5]. Diabetes mellitus (DM) is linked to an increased incidence of cardiovascular diseases such as coronary heart disease, heart failure, myocardial infarction (MI) and angina, which in turn lead to disability and death [6]. Atrial fibrillation (AF) is the most common cardiac arrhythmia in adults, with a current prevalence of 2-4% [7]. It is seen in diabetic patients, and prevalence rates are estimated to be at least twice as high as in non-diabetic subjects. In diabetic patients with hypertension, a common co-existing condition, the prevalence of AF is up to three times higher than in patients with hypertension or diabetes alone [7]. AF can remain undetected for months or years and is often undiagnosed or untreated until a stroke occurs. Therefore, a reliable, easy-to-apply tool for early detection of AF is crucial in clinical practice, especially in hypertensive and diabetic patients [8]. The electrocardiogram (ECG) is a practical, reliable and rapid tool that has been widely used to screen for cardiovascular disease, specifically cardiac arrhythmias [6]. Hypertension, hyperglycaemia, but also hypercholesterolaemia are well known risk factors strongly implicated in the occurrence of cardiovascular events [9]. Chronic hyperglycaemia in diabetics is likely to trigger and promote vascular complications by inducing several metabolic alterations, including increased formation of advanced glycation end products (AGEs) and increased production of reactive oxygen species (ROS) leading to cardiovascular remodelling and alteration of ion channels involved in the regulation of heart rate and rhythm

[10-11]. Current therapies, such as chemical drugs, are insufficient to cure or stop the progression of this disease with its multiple manifestations. In addition, numerous studies have found that the use of antihypertensive drugs to control high blood pressure in diabetics is associated with a higher risk of AF [5]. As a result, several laboratories are undertaking research into the treatment of hypertensive diabetics and the complications associated with this association [12]. These drugs although promising are very expensive, inaccessible and have many side effects. Plants are good sources of alternative or complementary medicines in the treatment of various diseases. Based on the WHO report, it is considered that about 75-80% of the world's population depends mainly on traditional formulations or medicines obtained from plant materials and products [13]. *Erythrina senegalensis* which is the subject of this work is a Fabaceae and used in traditional medicine in the treatment of many diseases including hypertension and diabetes. Furthermore, previous scientific work *in vivo* with *E. senegalensis* has revealed hypotensive, cardioprotective, hypoglycaemic and antioxidant properties [14]. These different effects are attributed to the multitude of compounds such as polyphenols, more specifically flavonoids, tannins and steroids present in the *E. senegalensis* extract [14]. So far, no work showing the effects of *E. senegalensis* on cardiovascular complications in hypertensive diabetic rats has been reported. The present work is conducted to evaluate the activity of the aqueous extract of *E. senegalensis* on cardiovascular function in diabetic hypertensive rats. Specifically, the effects on cardiac electrical activity, oxidative status, aortic and cardiac ultrastructure will be determined.

2 Materials and Methods

2.1 Plant collection and extract preparation

The stem barks of *Erythrina senegalensis* were harvested at Bafia-goufan (Mbam and Inoubou Department), Central Region of Cameroon in June 2017. The plant was authenticated at the National Herbarium of Cameroon where voucher specimen N° 13752 SRF Cam has been deposited. The barks were shade dried and crushed. Aqueous extract was prepared according to the protocol described by Bilanda *et al* [14]. Briefly, the powder (250 g) was added to 3 L of distilled and allowed to macerate at room temperature for 24 h and then filtered with Wattman No. 3 filter paper. The filtrate obtained was evaporated in an oven (40-45 °C) and a mass of 26.78 g of dry extract was obtained, giving a yield of 10.71 %.

2.2 Drugs and chemicals

Some drugs and some biochemicals used in this experiment were purchased from Sigma Chemical Company (St. Louis, MO, USA); others from LABKIT (ESPAGNE). The chemicals were of analytical grade.

2.3 Animals

Eight to ten weeks old male albinos Wistar rats, weighing between 180 and 200 g were used. The animals were raised in the animal house of the Laboratory of Animal Physiology of the University of Yaounde I (5 animals per cage). They were kept at room temperature under a natural light cycle (12/12) with adequate ventilation and had free access to tap water as well as a standard animal diet. Prior authorization for the use of laboratory animals in this study was obtained from the Cameroon National Ethics Committee (Reg. Nº. FWA-IRD 0001954).

2.4 Hypertension and diabetes Induction and treatment of animals

Nine to twelve weeks aged normoglycaemic rats weighing between 200-250 g, received a daily administration of 15% sucrose followed by 40° alcohol by gavage at doses of 1.5 g/kg and 5 g/kg, respectively, for 6 weeks. Another group (7 rats) received distilled water at a dose of 10 mg/kg (normal group). On day 43, the hypertensive animals received one intravenous (dorsal vein of the penis) injection (it) of streptozotocin (STZ) solution at a dose of 35 mg/kg after 12 hours of non-hydrous fasting. At the same time, those in the normal group received 0.9% NaCl (it). The blood glucose levels of these rats were determined two weeks after STZ administration using a glucometer and ACCU-CHEK ® Performer strips. Animals with blood glucose level from 126 mg/dL and above were considered diabetic and left for two additional weeks to settle complications.

Hypertensive diabetic rats continued to receive alcohol (5 g/kg) and sucrose (1.5 g/kg) and were divided into 5 groups of 5 animals each and received additional treatment for 4 weeks as follows: a hypertensive diabetic control group (HDC) received distilled wate; a positive control group (MNC) received metformin (200 mg/kg) plus nifedipine (10 mg/kg); three test groups (ES 50, ES 100 and ES 300) received aqueous extract of *E. senegalensis* at doses of 50, 100 and 300 mg/kg respectively. The group receiving distilled water (10 mL/kg) continued to receive it and made up the normal control group (NC). The blood glucose level of the animals was taken at the end of each. During the treatment period, all animals continued to receive sucrose and alcohol except those of the normal control group.

2.5. Haemodynamic parameters and electrocardiograph recording

At the end of the treatment, haemodynamic parameters and electrocardiogram (ECG) were recorded by the direct method as described by Bilanda *et al*. [14]. Briefly, each rat was anaesthetised by intraperitoneal injection of urethane (1.5 g/kg). The trachea was exposed and cannulated to facilitate spontaneous breathing. Blood pressure and heart rate were measured from the right carotid artery via an arterial cannula connected to a pressure transducer coupled to a Biopac Student Lab hemodynamic recorder (MP35) and a computer. A 30-minute equilibration period was observed before each measurement. The ECG was evaluated by cancelling the blood pressure and heart rate curve at 0.4 second/division.

2.6 Biochemical analyses

After hemodynamic recording, animal was sacrificed by the disruption of the carotid artery, the aorta and the heart were removed and weighed. One part of each organ was crushed in the Mac Even solution and centrifuged at 3000 rpm. The supernatant was used as homogenate (20%) for the determination of some biochemical parameters. Cardiac total cholesterol (TC), triglycerides (TG), and HDL Cholesterol (HDL-C) levels were assessed using commercial diagnostic kits (LABKIT, UK). The levels of LDL-Cholesterol (LDL-C) and Atherogenic index (AI) were calculated using the following formula: LDL-Chol (mg/dL) = Chol - (TG/5) - HDL-Chol Youmbissi et *al*. [15] respectively. Similarly, the cardiac levels of sodium, calcium, potassium, magnesium and chlorine were assessed using commercial diagnostic kits LABKIT. Tissue protein concentration was assayed according to Gornall *et al*. [16] Catalase was determined according to Sinha [17] whereas glutathione reduced and superoxide dismutase were determined using the method of Ellman [18], Misra and Fridovish [19] respectively. The end product of lipid peroxidation (malondialdehyde, MDA) was determined using the procedure of Wilbur *et al*. [20] and the Nitrite concentration (an indirect measurement of NO synthesis) was assayed using the method describe by Green *et al*. [21].

2.7 Histological analyses

The aorta and heart after fixation (1 week) in 10% buffered formaldehyde were trimmed and dehydrated in increasing gradient alcohol (70%, 80%, 90% and 100% (3 baths)). The tissues were then clarified in 2 xylene baths (1.5 hrs per bath) and impregnated in liquid paraffin at 60°C (for 5 hrs). 5 µm sections of paraffin-embedded aorta and heart tissue after haematoxylin-eosin's staining were examined with a microscope and media sizes of aorta were assessed.

2.8 Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.0.1. All data were analysed by one-way analysis of variance (ANOVA) followed by the Tukey post hoc test and expressed as a mean ± standard error of the mean (SEM). The difference was considered significant at $p < 0.05$.

3 Results

3.1 Effects of *E. senegalensis* extract on glycaemic evolution

Fig 1 shows the evolution of blood glucose levels, before and during the treatment with the aqueous extract of *E. senegalensis* or metformin + nifedipine (MN). The results obtained show a significant increase in blood glucose level in the different groups after hypertension and diabetes induction (daily administration of sucrose followed by alcohol and intravenous injection of streptozotocin) as well as the first day of treatment (D1). That increase was of 26.83% ($p < 0.001$) and 234.13% ($p < 0.001$) respectively as compared to normal group. During the treatment, there was a significant increase in blood glucose levels in the diabetic hypertensive control group of 244.74% ($p<0.001$), 244.59% ($p<0.001$), 272.81% ($p<0.001$) and 200.71% ($p<0.001$) respectively still as compared to the normal group. At the end of the first week of treatment, the group receiving metformin plus nifedipine (MNC) treatment as well as those receiving the extract at different doses showed a significant decrease in blood glucose levels as compared to diabetic hypertensive control group. The values were of 23.76% ($p < 0.001$), 19.15% ($p < 0.001$), 27.30% ($p < 0.001$) and 30.50% ($p < 0.001$) respectively lower. Similarly, at end of the second week, the MNC group as well as those receiving the extract at the different doses showed a significant decrease in blood glucose levels as compared to the diabetic hypertensive control (HDC) group with the respective percentages of 29.41% ($p < 0.001$), 32.55% ($p < 0.001$), 30.20% ($p < 0.001$) and 38.82% ($p < 0.001$). At the end of the third week, the MNC group as well as those receiving the extract at different doses showed a significant decrease in blood glucose levels compared to HDC group of 35.38% ($p < 0.001$), of 59.57% ($p < 0.001$), of 57.76% ($p < 0.001$) and of 50.54% ($p < 0.001$) respectively. The MNC group as well as those receiving the extract at the different doses, showed, at the fourth week, a significant decrease in blood glucose levels as compared to HDC group at the percentages of 35.38% ($p < 0.001$), of 59.57% ($p < 0.001$), of 57.76% ($p < 0.001$) and of 50.54% ($p < 0.001$). At the end of week 4, the extract at the different doses brought the blood glucose values close to those of the normal control group.

Fig 1. Effects of *E. senegalensis* extract on blood sugar development

Each point represents the mean ± s.e.m; n = 5; [1]$p < 0.05$, [2]$p < 0.01$, [3]$p < 0.001$: significant differences from normal controls; [c]$p < 0.001$: significant differences from diabetic hypertensive controls. STZ: Streptozotocin. D: Day, W: Week.

3.2 Effects of aqueous extract of *E. senegalensis* on some haemodynamic parameters

Table 1 shows the effects of aqueous extract of *E. senegalensis* on blood pressure and heart rate after 4 weeks of treatment. A significant increase ($p < 0.001$) in systolic (SBP), diastolic (DBP), mean (MAP) and pulsatile (PP) blood pressure and heart rate (HR) was observed in hypertensive diabetic control (HDC) group as compared to the normal control (NC) group. This increase was of 34.78%, of 38.05%, of 37.40%, of 27.88% and of 25.00% respectively. The administration of metformin + nifedipine (MN) treatment as well as the extract at the different doses (ES50, ES100 and ES300 respectively) resulted in a significant decrease in these different parameters as compared to HDC group. This decrease was of 14.19% ($p < 0.001$), of 20.00% ($p < 0.001$), of 21.29% ($p < 0.001$) and of 23.23% for PAS respectively, of 14.64% ($p < 0.01$) and of 25.11% ($p < 0.001$) for PAD in ES100 and ES 300; of 8.70% ($p < 0.05$), of 15.30% ($p < 0.001$), of 18.00% ($p < 0.001$) and of 24.87% ($p < 0.001$) for MAP respectively; of 30.00% ($p < 0.001$), of 29.34% ($p < 0.001$), of 27.54% ($p < 0.001$) and of 24.26% ($p < 0.001$) for PP respectively; of 14.93% ($p < 0.001$), of 19.15% ($p < 0.001$), of 29.86% ($p < 0.001$) and of 21.69% ($p < 0.001$) for CF respectively. The plant extract at the different doses as well as the reference treatment reduced the blood pressure and heart rate to values close to those of the normal control group.

Table 1. Effects of aqueous extract of *E. senegalensis* on blood pressure, pulse pressure and heart rate

	NC	HDC	MNC	ES 50	ES 100	ES 300
SBP (mmHg)	115.00±2.59	155.00±2.20^{3}	133.00±1.62^{3c}	124.00±1.51^{1c}	122.00±1.23^{c}	119.00±2.28^{c}
DBP (mmHg)	67.80±1.23	93.60±1.11^{3}	90.60±2.61^{3}	83.90±3.44^{3}	79.90±2.08^{1b}	70.10±2.84^{c}
MAP (mmHg)	83.70±1.05	115.00±1.15^{3}	105.00±1.70^{3a}	97.40±3.73^{3c}	94.30±1.13^{1c}	86.40±1.79^{c}
PP (mmHg)	47.70±0.61	61.00±1.45^{3}	42.70±2.88^{c}	43.10±1.85^{c}	44.20±1.47^{c}	46.20±2.09^{c}
FC (BPM)	284.00±5.40	355.00±5.91^{3}	302.00±6.26^{c}	287.00±4.81^{c}	249.00±4.17^{2c}	278.00±6.49^{c}

Each value represents the mean ± s.e.m; n = 5; $^{1}p < 0.05$, $^{2}p < 0.01$, $^{3}p < 0.001$: significant differences from normal control group; $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$: significant differences from hypertensive diabetic control group.

3.3 Effects of *E. senegalensis* extract on the electrocardiogram

Fig 2 summarises the effects of *E. senegalensis* extract on the electrocardiogram (ECG), it was observed in the normal control group (NC) a normal ECG curve with different segment waves, well identifiable intervals, regular cardiac cycles. On the other hand, in the hypertensive diabetic control group (HDC), a decrease in the amplitude of the different waves was noted, as well as an abnormal sequence of these waves. Similarly, a decrease in the duration between two consecutive beats (RR) and irregular cardiac cycles were observed, indicating atrioventricular defibrillation. The administration of the plant extract as well as the MN treatment improved the ECG of the animals in these groups as compared to HDC group.

Fig 2. Effects of *E. senegalensis* extract on the electrocardiograph at 0.4 seconds/div

P: Atrial depolarization, **T**: Ventricular repolarization, **QRS**: Ventricular depolarization, **RR**: Cardiac revolution; **NC**: Normal control rats, **HDC**: Hypertensive diabetic control rats, **TMN**: Hypertensive diabetic control rats receiving metformin and nifedipine at doses of 200 and 10 mg/kg, respectively, **ES 50, 100 and 300**: Hypertensive diabetic rats receiving *E. senegalensis* extract at doses of 50, 100 and 300 mg/kg, respectively.

3.4 Effects of *E. senegalensis* extract on some electrocardiogram parameters

3.4.1 Effects of *E. senegalensis* extract on the duration of some ECG parameters

Table 2 shows the effects of *E. senegalensis* extract on the duration of some ECG waves and segments. In the hypertensive diabetic control (HDC) group, a significant ($p<0.01$) decrease of 15.29% in atrioventricular conduction time (AVT) was observed as compared to the normal control (NC) group. The administration of metformin + nifedipine (MN) as well as the extract at different doses (ES50, ES100, ES300 respectively) resulted in a significant ($p < 0.001$) increase in the duration of the PR interval as compared to HDC group. This increase was of 34.43%, of 47.56%, of 57.38% and of 37.71% for the MNC, ES 50, ES 100 and ES 300 groups respectively as compared to HDC group.

There was also a significant decrease ($p<0.05$) in the duration of ventricular contraction (QRS complex) in the THD group as compared to the NC group. This decrease was of

30.29%. The plant extract (50 mg/kg) treatment led to a significant ($p < 0.05$) increase in the duration of ventricular depolarisation as compared to diabetic hypertensive animals.

The diabetic hypertensive animals as compared to the normal control animals showed a significant ($p < 0.001$) decrease of 29.57% in the cardiac revolution time (RR segment). Dual therapy with metformin and nifedipine as well as plant extract at different doses significantly ($p < 0.001$) increased the RR segment compared to the HRT group. This increase was of 40.16%, of 24.26%, of 41.07% and of 38.00% for the MNC, ES 50, ES 100 and ES 300 groups respectively as compared to HDC.

In HDC group as compared to NC animals, there was a significant ($p<0.01$) 38.12% decrease in the duration of ventricular activity (QT interval). The combination of metformin and nifedipine, as well as the extract at different doses, resulted in a significant ($p < 0.01$) increase in QT interval duration in these groups compared to HDC group. This increase was of 51.29%, of 44.42%, of 59.89% and of 44.42% for the MNC, ES 50, ES 100 and ES 300 groups as compared to HDC group.

There was a significant ($p<0.01$) decrease in ventricular repolarisation time (ST segment) of 26.27% in HDC group as compared to NC group. The combination of metformin and nifedipine as well as the plant extract (300 mg/kg) resulted in a significant increase in ventricular repolarisation time as compared to HDC animals. This increase was of 68.70% ($p < 0.001$) and of 33.74% ($p < 0.01$) for the MNC and ES 300 groups respectively.

Table 2. Effects of *E. senegalensis* extract on the duration of some ECG parameters

	NC	**HDC**	**MNC**	**ES 50**	**ES 100**	**ES 300**
PR (ms)	51.43±1.80	43.57±1.43$^{\mathbf{2}}$	58.57±0.92$^{\mathbf{2c}}$	64.29±1.30$^{\mathbf{3c}}$	68.57±1.43$^{\mathbf{3c}}$	60.00±1.09$^{\mathbf{3c}}$

QRS (ms)	23.57±2.68	16.43±1.01[1]	17.14±1.49[1]	22.86±1.01[a]	22.14±1.07	19.29±0.71
RR (ms)	217.10±5.96	152.90±3.91[3]	214.30±8.69[c]	190.00±5.56[1c]	215.70±2.02[c]	211.00±2.10[c]
QT (ms)	78.33±3.33	48.47±1.78[2]	73.33±5.87[a]	70.00±4.75[a]	77.50±6.55[b]	70.00±2.58[a]
ST (ms)	63.75±3.19	47.00±1.52[2]	79.29±5.04[2c]	57.86±2.29	59.29±1.47	62.86±2.47[b]

Each value represents the mean ± s.e.m; n = 5; [1]$p < 0.05$. [2]$p < 0.01$. [3]$p < 0.001$: significant differences from normal control group; [a]$p < 0.05$. [b]$p < 0.01$. [c]$p < 0.001$: significant differences from hypertensive diabetic control group.

3.4.2 Effects of *E. senegalensis* extract on duration of atrial depolarisation (P-wave) and ventricular repolarisation (T-wave)

Fig 3 shows the effects of *E. senegalensis* on the duration of atrial depolarisation and ventricular repolarisation. A significant decrease in P- and T-wave duration of 32.36% ($P < 0.05$) and of 31.25% ($P < 0.01$) respectively was observed in hypertensive diabetic control (HDC) animals as compared to NC animals. The administration of the metformin and nifedipine mixture as well as the plant extract at different doses resulted in a significant increase in P-wave duration as compared to HDC animals. This increase was of 69.57% ($P<0.001$), of 60.86% ($P<0.01$) and of 78.27% ($P<0.001$) in the ES 50, ES 100 and ES 300 groups, respectively (Fig 3A). For the T-wave, this increase was of 31.25% ($p < 0.01$), of 55.84% ($p < 0.001$) and of 42.99% ($p < 0.01$) for the T-wave in the TMN, ES 50 and ES 100 groups respectively (Fig 3B).

Fig 3. Effects of *E. senegalensis* extract on the duration of atrial depolarisation (A) and ventricular repolarisation (B).

Each bar represents the mean ± s.e.m; n = 5; $^{2}p < 0.01$. $^{3}p < 0.001$: significant differences from normal control group; $^{b}p < 0.01$: significant differences from diabetic hypertensive control group.

3.5 Effects of *E. senegalensis* extract on the amplitude of atrial, ventricular depolarisation and ventricular repolarisation

The effects of aqueous extract of *E. senegalensis* on the amplitude of atrial depolarisation (P wave), ventricular depolarisation (QRS complex) and ventricular repolarisation (T wave) are summarised in Fig 4. In hypertensive diabetic animals as compared to normal animals there was a significant decrease in the amplitude of the P-wave ($p < 0.001$), QRS complex ($p < 0.05$) and P-wave ($p < 0.05$) of 42.47%, of 31.28% and of 27.94%. The combination of metformin and nifedipine as well as plant extract at different doses resulted in a significant increase in P-wave amplitude as compared to hypertensive diabetic animals. The values were of 60.64% ($p<0.001$), of 66.50% ($p<0.001$), of 58.59% ($p<0.001$) and of 38.07% ($p<0.05$) lower in the MNC, ES 50, ES 100 and ES 300 groups, respectively (Fig 4A). For ventricular depolarisation, the increase was 1.36-fold ($p<0.001$) and 63.59% ($p<0.01$) in the MNC and ES 100 groups respectively (Fig 4B). The amplitude of repolarisation increased by 54.66% ($p<0.001$), by 47.03% ($p<0.01$) and by 78.19% ($p<0.001$) for ventricular repolarisation in the MNC, ES 50 and ES 100 groups respectively compared to HDC group (Fig 4C).

Fig 4. Effects of *E. senegalensis* extract on P, T waves and QRS complex amplitudes.

Each value represents the mean ± s.e.m; n = 5; [1] $p < 0.05$. [3] $p < 0.001$: significant differences from normal control group; a $p < 0.05$. [c] $p < 0.01$. [c] $p < 0.001$: significant differences from hypertensive diabetic control group.

3.6 Effects of *E. senegalensis* extract on some parameters of cardiac lipid profile

The effects of aqueous *E. senegalensis* extract on cardiac total cholesterol, HDL-cholesterol, triglycerides and atherogenic index are summarised in Table 3. The results show in comparison with the normal control group an increase in total cholesterol, triglyceride and atherogenic index levels. This increase was of 54.16% ($p < 0.01$), of 146.15% ($p < 0.001$) and of92.00% ($p < 0.001$) respectively; a significant decrease ($p < 0.01$) in HDL-cholesterol levels of 37.42%. The administration of the combination of metformin-nifedipine and the extract at different doses resulted in a significant decrease in the concentration of total cholesterol. triglycerides and the atherogenic index. This significant decrease ($p < 0.001$) was of 50.09% of44.87%, of 50.89% and of 42.48% for total cholesterol; of 51.19% of 58.18 of. 39.73% and of 61.31% for triglycerides; of 55.65%, of 64.88%, of 55.36% and of 55.95% for the atherogenic index respectively as compared to HDC group. The extract increased HDL-cholesterol levels by 123.39%, by 61.86% and by 122.68% in the groups receiving the extract at 50. 100 and 300 mg/kg respectively as compared to HDC group.

Table 3. Effects of *E. senegalensis* extract on some parameters of the cardiac lipid profile

	NC	HDC	MNC	ES 50	ES 100	ES 300
T- Cho (mg/dL)	0.73±0.09	1.13±0.07^{2}	0.56±0.02^{c}	0.62±0.06^{c}	0.56±0.10^{c}	0.65±0.03^{c}
HDL-C (mg/dL)	0.47±0.01	0.29±0.04^{2}	0.38±0.01	0.65±0.03^{2c}	0.47±0.03^{b}	0.65±0.01^{2c}
TG (mg/dL)	0.27±0.02	0.67±0.01^{3}	0.33±0.06^{c}	0.28±0.03^{c}	0.41±0.05^{c}	0.26±0.02^{c}
Atherogenic Index	1.45±0.11	3.36±0.12^{3}	1.25±0.08^{c}	1.08±0.04^{1c}	1.10±0.14^{c}	0.94±0.20^{c}

Each value represents the mean ± s.e.m; n = 5; ^{2} $p < 0.01$. ^{3} $p < 0.001$: significant differences from normal control group; ^{b} $p < 0.01$. ^{c} $p < 0.001$: significant differences from diabetic hypertensive control group. T- Cho: Total-cholesterol, HDL-C: High-density lipoprotein cholesterol, TG: Triglycerides.

3.7 Effects of *E. senegalensis* extract on the cardiac ionogram

Table 4 shows the effects of *E. senegalensis* extract on the cardiac levels of some ions. The results show in the hypertensive diabetic control (HDC) group as compared to the normal control (NC) group a significant increase in the concentrations of sodium (Na^{+}), potassium (K^{+}), calcium (Ca^{2+}) chlorine (cl^{-}) ions and a significant decrease in magnesium (Mg^{2+}) ion. This increase was of 54.91% ($p < 0.001$), of 45.87% ($p < 0.05$), of 22.73% ($p < 0.05$), of 40.27% ($p < 0.01$) and of 51.08% ($p < 0.001$) respectively. The administration of MN treatment as well as the extract at different doses resulted in a significant decrease in the level of Na^{+}, K^{+}, Ca^{2+}, cl^{-} ions and a significant increase in Mg^{2+} ions as compared to HDC group. This decrease was of 21.39% ($p < 0.05$), of 25.35% ($p < 0.01$), of 32.28% ($p < 0.001$) and of 28.91% ($p < 0.001$) for sodium; of 35.85% ($p < 0.01$), of 38.81% ($p < 0.01$), of 32.08% ($p < 0.05$) and of 30.19% ($p < 0.05$) for potassium; of 29.63% ($p < 0.001$), of 22.90% ($p < 0.001$), of 38.46% ($p < 0.001$) and of 35.19% ($p < 0.001$) for calcium respectively as compared to HDC group. The values were lower by 38.76% ($p < 0.001$), by 30.62% ($p < 0.01$), by 43.06% ($p < 0.001$) and by 32.54% ($p < 0.01$) for chlorine respectively and higher by 32.46% ($p < 0.05$). by 75.95% ($p < 0.001$) and by 87.76% ($p < 0.001$) respectively in MNC, ES50 and ES100groups. Both the plant extract and MN treatments brought the ion levels close to the values of the normal control group.

Table 4. Effects of *E. senegalensis* on the levels of some ions in the heart

	NC	HDC	MNC	ES 50	ES 100	ES 300
[Na^+] (mg/dL)	326.00±17.20	505.00±25.60^{3}	397.00±18.20^{a}	377.00±19.50^{b}	342.00±30.80^{c}	359.00±13.30^{c}
[K^+] (mg/dL)	10.90±0.80	15.90±0.71^{1}	10.20±0.91^{b}	9.73±0.60^{b}	10.80±1.20^{a}	11.10±1.36^{a}
[Ca^{2+}] (mg/dL)	13.20±0.52	16.20±0.46^{1}	11.40±0.78^{c}	12.49±0.47^{c}	9.97±0.73^{2c}	10.50±0.40^{1c}
[Mg^{2+}] (mg/dL)	10.20±0.20	4.99±0.16^{3}	6.61±0.42^{3a}	8.78±0.32^{c}	9.37±0.30^{c}	5.60±0.56^{3}
[Cl^-] (mg/dL)	14.90±1.03	20.90±1.06^{2}	12.80±0.71^{c}	14.50±1.25^{b}	11.90±0.60^{c}	14.10±1.32^{b}

Each value represents the mean ± s.e.m; n = 5; [1] $p < 0.05$. [2] $p < 0.01$. [3] $p < 0.001$: significant differences from normal control group; [a] $p < 0.05$. [b] $p < 0.01$. [c] $p < 0.001$: significant differences from hypertensive diabetic control group.

3.8 Effects of *E. senegalensis* extract on relative heart and aorta weight

The results show in the hypertensive diabetic control (HDC) group as compared to the normal control (NC) group a significant increase in the relative weight of the heart and aorta. This increase was of 72.55% ($p < 0.001$) and of 68.24% ($p < 0.05$) respectively. The administration of the MN treatment as well as the extract at different doses resulted in a significant ($p < 0.001$) decrease in the relative heart weight of 29.01%, of 24.01%, of 23.86% and of 26.82% respectively. In the aorta of the group that received the extract at the dose of 50 mg/kg (ES50) as compared to HDC group, the value was by 36.00 % lower. The plant extract as well as MN treatment reduced the relative weight of the heart and aorta close to those of the normal control. (Table 5).

Table 5. Effects of *E. senegalensis* extract on relative heart and aorta weight

	NC	HDC	MNC	ES 50	ES 100	ES 300
Heart	0.255±0.013	0.440±009^{3}	0.312±0.011^{2c}	0.334±0.010^{3c}	0.335±0.011^{3c}	0.322±0.009^{2c}
Aorta	0.030±0.002	0.050±0.002^{1}	0.040±0.004	0.032±0.005^{a}	0.040±0.005	0.041±0.002

Each value represents the mean ± s.e.m; n = 5; [1] $p < 0.05$. [2] $p < 0.01$. [3] $p < 0.001$: significant differences from normal control group; [a] $p < 0.05$. [c] $p < 0.001$: significant differences from hypertensive diabetic control group.

3.9. Effects of *E. senegalensis* extract on some cardiac tissue parameters

Fig 5 shows the effects of aqueous extract of *E. senegalensis* on tissue levels of reduced glutathione (GSH). malondialdehyde (MDA), nitrite, protein, catalase and superoxide dismutase (SOD) activity.

A significant ($p<0.001$) decrease in GSH levels in the aorta and heart was observed in the hypertensive diabetic control (HDC) group as compared to the normal control group. This decrease was of 39.05% and of 36.48% respectively. The administration of the extract at different doses resulted in a significant ($p<0.001$) increase in these levels as compared to the HDC group. This increase was of 57.04%, of 44.37% and of 34.51% for the aorta respectively. The values were by 37.10%, by 28.81%, by 42.00% and by 59.13% in the heart with MN treatment or the extract at different doses respectively as compared to HDC group (Fig 5A).

A significant ($p < 0.001$) increase was observed in MDA levels in HDC group as compared to the normal group. This increase was by 75.60% and by 79.05% in the aorta and heart respectively. The MN treatment as well as the extract at the respective doses resulted in a significant ($p<0.001$) decrease in MDA levels in the aorta by 31.53%, by 44.86%, by 48.47% and by 49.39%. The values were lower in the heart by 21.81%, by 28.72%, by 39.36% and by 34.04% respectively as compared to HDC group (Fig 5B).

The HDC group as compared to the NC group showed a significant decrease in catalase and SOD activity. This decrease was of 47.19% ($p< 0.01$) and of 30.19% ($p < 0.001$) for catalase. For SOD, the values were of 32.41% ($p < 0.001$) and of 54.24% ($p < 0.001$) in the aorta and heart respectively. The MN treatment as well as the extract at different doses resulted in a significant increase in this activity. This increase was of 79.52% ($p < 0.01$) and of 62.69% ($p < 0.05$) respectively in the aorta for the groups receiving the extract at doses of 100 and 300 mg/kg and by 33.78% ($p < 0.01$), by 25.23% ($p < 0.05$) and by 46.40% ($p < 0.001$) respectively in the heart in the groups receiving the 50, 100 and 300 mg/kg extract. Both MN treatment and the extract at the different doses reduced catalase activity close to that of the NC group (Fig 5C). Similarly, a significant increase in superoxide dismutase activity was observed in the groups treated with MN or the extract at the different doses. The values were higher by 28.91% ($p < 0.01$) and by 33.67% ($p < 0.01$) respectively in the aorta of the groups treated with the extract at the doses of 100 and 300 mg/kg; by 21.90% ($p < 0.05$), by 93.80%

($p < 0.001$) and by 79.20% ($p < 0.001$) respectively in the heart of the groups treated with the extract at the doses of 50, 100 and 300 mg/kg (Fig 5D) as compared to HDC group.

Fig 5E shows a significant ($p<0.001$) decrease in nitrite levels in the aorta and heart of the hypertensive diabetic control (HDC) group as compared to the normal control (NC) group. This decrease was of 38.13% and of 33.65% respectively. The 100 and 300 mg/kg extract treatment significantly ($p<0.01$) increased nitrite levels in the aorta by 41.28% and by 47.09% respectively, in the heart by 35.46% ($p<0.05$), by 53.55% ($p<0.001$), by 48.23% ($p<0.001$) and by 42.91% ($p<0.01$) with MN treatment or extract (50, 100 and 300mg/kg) respectively.

The hypertensive diabetic control (HDC) group showed a significant ($p < 0.01$) increase in protein levels in the aorta and a significant ($p < 0.01$) decrease in the heart as compared to the control group. The increase was of 51.16% and the decrease by 37.97%. The treatment with the extract at different doses as compared to HDC group resulted in a significant decrease in the amount of protein in the aorta. This decrease was of 28.68% ($p < 0.05$), of 36.37% ($p < 0.01$) and of 37.03% ($p < 0.001$) respectively in the aorta of the groups treated with the extract at doses of 50. 100 and 300 mg/kg, a significant increase in the protein level in the heart of the groups treated with the extract at doses of 100 and 300 mg/kg (Fig 5F) as compared to HDC group.

Fig 5. Effects of *E. senegalensis* extract on some tissue parameters

Each bar represents the mean ± s.e.m; n = 5; [1]$p < 0.05$. [2]$p < 0.01$. [3]$p < 0.001$: significant differences from normal control group. [a]$p < 0.05$. [b]$p < 0.01$. [c]$p < 0.001$: significant differences from hypertension diabetic control group.

3.10 Effects of aqueous extract of *E. senegalensis* on the structure of the aorta and heart

Fig 6 shows the effects of *E. senegalensis* on the structure of the aorta and heart of hypertensive diabetic rats. There was a significant ($p<0.001$) increase the media size in the

hypertensive diabetic group compared to the normal control group. This increase was of the order of 74.61%. The MN treatment as well as the plant extract significantly ($p<0.001$) decreased the media size by 29.14%, by 31.23%, by 37.39% and by 38.00% respectively (Fig 6A, 6B). Disorganisation of cardiac muscle fibres was also observed. Both the plant extract and MN treatment as compared to HDC group improved the structure of the myocardium and the aorta (Fig 6C).

Fig 6: Microarchitecture of the aorta and heart (HE. 100X)

Aorta: I = Intima, M = Media, Ad = Adventitia, **Heart**: Co = Cores, Mf = Muscle fibers, Li: Leucocytes infiltration.

4 Discussion

Cardiovascular complications are among the main causes of morbidity and mortality in diabetic hypertension [6]. Strict control of blood glucose and blood pressure has been shown to limit these various complications in diabetic hypertensive patients [8]. The objective of this study was to evaluate the effects of aqueous extract of *E. senegalensis* on cardiovascular complications in a hypertensive diabetic rat model. Administration of sucrose and alcohol followed by a single intravenous injection of streptozotocin resulted in an increase in blood glucose, systolic, diastolic and mean blood pressure and heart rate. These results are in agreement with those of Bilanda *et al.* [14] who observed the same changes in blood glucose and haemodynamic after the administration of alcohol plus sucrose and streptozotocin. Alcohol plus sucrose administration is a well-established animal model of hypertension [14-22]. Streptozotocin on the other hand is known to destroy β-pancreatic cells, leading to increased blood glucose and diabetes. The reduction in blood pressure and blood glucose with *E. senegalensis* can be attributed to its antihypertensive and antidiabetic properties [14]. Hypertension is usually accompanied by an increase in heart rate (tachycardia). This was demonstrated in the present study by the increase in relative heart weight observed in hypertensive diabetic rats. When the heart beats rapidly, it pumps blood less efficiently, thus reducing the blood supply to the heart. The tachycardia also leads to increased work and oxygen requirements of the heart muscles. In addition, an increase in the atherogenic index in diabetic hypertensive rats associated with an increase in the size of my aortic media was noted. This cardiovascular remodelling would be responsible for the hypertrophy of the aorta and the heart through the stimulation of mitogenic pathways, the accumulation of collagen at the origin of fibrosis [23]. This structural remodelling of ventricular wall components due to their adaptive response to physiological alterations due to hypertension and diabetic-induced myocardial injury is another reason for cardiac hypertrophy [24]. Treatment with *E. senegalensis* may controlled the workload of the heart by decreasing heart rate and then reduce its weight, thus safeguarding the myocardium of hypertensive diabetic rats through its antihypertensive and antidiabetic properties. The increased heart rate in hypertensive diabetics may be related to an abnormality in the electrical activity of their hearts. Indeed, the PR interval of the ECG represents the AV

conduction time, while the QRS represents the total duration of ventricular depolarisation and their alteration reflects an abnormality in cardiac function. The QT interval represents the period of ventricular repolarisation and is determined by Na^+ and Ca^{2+} inflow and K^+ and Cl^- outflow currents [25]. In addition, the QT interval represents the period of electrical systole which is a method to determine the functional integrity of the myocardium [26]. Some authors demonstrate a strong involvement of hypercholesterolemia in atrioventricular conduction time, atrioventricular depolarisation and ventricular repolarisation [9-10]. The reduction in time and amplitude of these waves and segments resulted in a pathological electrocardiogram (ECG). In fact, although morphological and functional alteration of very small vessels is the main pathophysiological feature of diabetes, myocardial dysfunction independent of coronary alterations is also observed in diabetes [27]. Several mechanisms such as migration/infiltration of epicardial adipocytes between atrial and ventricular myocytes have been implicated in the creation of electrically inert zones, which confound and accelerate electrical conduction [9-10]. All these mechanisms act in isolation and/or synergistically for atrioventricular electrical and anatomical remodelling [6]. The regulation of heart rate by the electrical activity of *E. senegalensis* may be due to its blockade of angiotensin II-like receptors, which have been shown to reduce type I collagen fibre synthesis and stimulate type I collagen fibre degradation. Thus, *E. senegalensis* may be able to decrease myocardial collagen content and regress fibrosis independently of its antihypertensive effect [28]. It has been suggested that endothelial cell dysfunction is the cause of cardiovascular damage induced by diabetes and hypertension; and this dysfunction is represented by an increase in endothelial adhesiveness with the release of adhesion molecules responsible for leukocyte migration into the vascular wall, where leukocytes transform into inflammatory foam cells evident in atherosclerotic fatty streaks [29]. These cardiovascular alterations were noted in the present study by the increased aortic wall and leukocyte infiltration in the hearts of hypertensive diabetic rats. Diabetic-induced hypertension and hyperlipidaemia, oxidation and stress appear to be the major contributors to abnormal membrane-related enzyme activities leading to cardiac dysfunction. Hypercholesterolaemia, hypertriglyceridemia, low HDL-cholesterol as well as high LDL-cholesterol concentration in the heart has been observed in the present work. Lipids play an important role in cardiovascular disease, not only in the development of atherosclerosis, but also by modifying the composition, structure and stability of cell membranes. High concentrations of triglycerides and LDL-cholesterol are involved in the pathogenesis of atherosclerosis through oxidation which progressively leads to blockage and narrowing of the artery lumen [30]. The accumulation of cholesterol in the heart and the

atherogenic index expose the heart and aorta to oxidative damage [29-30]. Normalization of blood glucose and blood pressure could be one of the important mechanisms used by *E. senegalensis* to protect the hypertensive diabetic's heart [8]. Furthermore, the oxidative stress observed in the aorta and heart of hypertensive diabetic rats is due to the direct action of alcohol and sucrose on the production and bioavailability of NO, which is a potent vasodilator, through inhibition of eNOS [31]. On the other hand, chronic hyperglycaemia is likely to activate several metabolic pathways, including the polyol pathway of hexosamines and protein kinase C, which are strongly implicated in endothelial dysfunction and in the installation of oxidative stress by a decrease in the expression of NADPH, which is responsible for a failure of the antioxidant defence system and an increase in lipid peroxidation [32]. The decrease in catalase and SOD activity, GSH concentration, nitrites and the increase in MDA levels observed in diabetic hypertensive rats in this study confirm this hypothesis. Oxidative stress-inducing ROS production and NF-kB activation are induced by activation of the AGE receptor on the surface of endothelial cells, resulting in endothelial dysfunction [31]. The decrease in blood glucose, haemodynamic parameters and the improvement in oxidative status after treatment with the plant extract could be related to the presence of flavonoids whose antioxidant properties have already been demonstrated [11]. But also, to the insulin-mimetic effect that flavonoids could have by stimulating the transport (GLUT4) and the use of glucose by the cells favouring hypoglycaemia [32-34]. Cardiac myocytes are not only dependent on the pacemaker mechanism, but also on the activity of ion channels found on the surface of their membranes [25]. The function of these proteins can be altered by glycation, but also by the action of proteases most often found in diabetics [35]. In the present study, an increase in Na^+, K^+, Ca^{2+}, Cl^- and a decrease in Mg^{2+} were noted. These results could be due to the abnormalities in Na^+/K^+ and Ca^{2+}-ATPase pump activity well documented in cardiac dysfunction in diabetic hypertension [36]. The extract of *E. senegalensis* by its antihypertensive, hypoglycaemic and antioxidant properties would have prevented the glycation of these proteins and/or inhibited the action of proteases while protecting the membranes of cardiac myocytes against peroxidation thus improving the fluidity of the membrane and the activity of ion channels [37].

Conclusion

At the end of the present work, the main aim of which was to evaluate the effects of the aqueous extract of *E. senegalensis* on cardiovascular complications in hypertensive diabetic rats. The aqueous has preserved the heart structure electrical and mechanical activity, thanks

to its regulation of glycaemia, its antihypertensive, hypolipidemia and antioxidative properties. This in turn leads to the maintenance of membrane fluidity that preserved ions flow. These results would be due to the secondary metabolites present in the extract and would justify its ethnopharmacological use in the treatment of hypertension-diabetes comorbidity and their cardiovascular complications.

Références

1. Marieb E, Hoehn K. Anatomie et physiologie humaine. *Traduction de la 8è Edition Américaine. Edition du Renouveau pédagogique Inc*. 2016 ; 1274p. 683-771.

2. Stephenson S, Atkinson A, Kottas P, Perde F, Jafarzadeh F, Bateman M. High resolution 3-Dimensional imaging of the human cardiac conduction system from microanatomy to mathematical modeling. *Scientific reports*. 2017 ; 7 :7188. Doi : 10.1038/s41598-017-07694-8.

3. Zhang Y, Wang Y, Yanni1 J, Qureshi M, Logantha S, Kassab S, Boyett M, Gardiner N, Hong Sun H, Howarth F, Dobrzynski H. Electrical Conduction System Remodeling in Streptozotocin-Induced Diabetes Mellitus Rat Heart. *Frontiers in. Physiology*. 2019 ; 10 : 826. Doi : 10.3389/fphys.2019.00826.

4. Faden G, Faganello G, De Feo S, Berlinghieri N, Tarantini L, Di Lenarda A, Faggiano P, Cioffi G. The increasing detection of asymptomatic left ventricular dysfunction in patients with type 2 diabetes mellitus without overt cardiac disease: Data from the SHORTWAVE study. *Diabetes research and clinical practice*. 2013 ; 8 : 2-8.

5. Orgah J, He S, Wang Y, Jiang M, Wang Y, Orgah E, Duan Y, Zhao B, Zhang B, Han J, Zhu Y. Pharmacological potential of the combination of Salvia miltiorrhiza (Danshen) and Carthamus tinctorius (Honghua) for diabetes mellitus and its cardiovascular complications. *Pharmacological research*. 2020 ; 153 (2020) 104654.

6. Mashayekhi Sardoo H, Mohammadpour A, Mehri1 S, Kamali H, Sahebkar A, Imenshahidi M. Diabetes mellitus aggravates ranolazine-induced ECG changes in rats. *Journal of Interventional Cardiac Electrophysiology*. 2021 ; https://doi.org/10.1007/s10840-021-01016-9.

7. Santos I, Lotufo P, Brant L, Pinto-Filho M, Pereira A, Barreto S, Ribeiro A, Thomas G, Lip G, Bensenor I. Atrial fibrillation giagnosis using ECG records and self-report in the community: cross-sectional analysis from ELSA-Brasil. *Arquivos brasileiros de cardiologia*. 2021 ; 117 :426-434.

8. Abellana R, Gonzalez-Loyola F, Verdu-Rotellar JM, Bustamante A, Palà E, Clua-Espuny J, Montaner J, Pedrote A, Val-Garcia J, Segui D, Muñoz M. Predictive model for atrial fibrillation in hypertensive diabetic patients. *European journal of clinical investigation*. 2021 ; 10 : 1-8.

9. Lee H, So-Ryoung L, Eue-Keun C, Kyung-Do H, Seil O. Low Lipid Levels and High Variability are Associated with the Risk of New-Onset Atrial Fibrillation. *Journal of the American Heart Association*. 2019 ; 46 : 2-35.

10. El-Menyar A. Dysrhythmia and electrocardiographic changes in diabetes mellitus: pathophysiology and impact on the incidence of sudden cardiac death. *Journal of Cardiovascular Medicine*. 2006 ; 7 : 580-585.

11. Thirunavukkarasu M, Zhan L, Wakame K, Fujii H, Moriyama H, Bagchi M. Safety of oligonol, a highly bioavailable lychee-derived polyphenolic antioxidant, on liver. kidney and heart function in rats. *Toxicology Mechanisms and Methods*. 2012 ; 22 : 555-559.

12. Hamiduddin M, Siddiqui A, Waris A, Gazi J, Akhter A. Unani formulations for management of diabetes: An overview. *International Journal of Green Pharmacy*. 2018 ; 15 : 3-13.

13. Khan M, Rawat A, Khatoonc S, Hussaind M, Mishrab A, Negi D. *In vitro* and *in vivo* Antidiabetic Effect of Extracts of *Melia azedarach*, *Zanthoxylum alatum*, and *Tanacetum nubigenum*. *Integrative medicine research*. 2018 ; 21 : 2-21.

14. Bilanda D, Bidingha à Goufani R, Dzeufiet Djomeni D, Fouda Bekono Y, Ngapout Fifen R, Tcheutchoua Y, Owona P, Wouamba Njonte S, Tatchou Tanfack L, Dimo T, Kamtchouing P. Antihypertensive and antidiabetic activities of *Erythrina senegalensis* DC (Fabaceae) stem bark aqueous extract on diabetic hypertensive rats. *Journal of Ethnopharmacology*. 2019 ; 10 : 1-10.

15. Youmbissi J, Djoumessi S, Nouedoui C, Ndobo P, Meli J. Profil lipidique d'un groupe d'hypertendus camerounais noirs africains. *Médecine Afrique Noire*. 2001; 48: 305-314.

16. Gornall A, Bradwill C, David M. Determination of serum proteins by means of the biuret reaction. *Journal of Biology and Chemistry*. 1949; 77: 167-182.

17. Sinha K. Colorimetric essay of catalase. *Analyze Biochemistry*. 47: 389-394.

18. Ellman G. ' ' Tissue sulfhydryl group' ' . *Archives of Biochemistry and Biophysics.* 1959; 82: 70-77.

19. Misra H, Fridovich I. Determination of the level of superoxide dismutase in whole blood. *Yale University Press New Haven*. 1972 ; 166 : 101-109.

20. Wilbur K, Bernheim F, Shapiro O. ' ' The Thiobarbituric acid reagent as a test for the oxidation of unsaturated fatty acids by various agents' ' , *Archives of Biochemistry and Biophysics.* 1949; 24: 305-313.

21. Green L, Wagner D, Godowsky J, Skipper P, Wishnok J, Tannenbaum S. ' ' Analysis of nitrate, nitrite and [15N] nitrate in biological fluids' ' . *Analytical Biochemistry,*1982; 126: 131-138.

22. Dzeufiet P, Mogueo A, Bilanda C, Aboubakar O, Tedong L, Dimo T, Kamtchouing P. Antihypertensive potential of the aqueous extract which combine leaf of *Persea americana* Mill. (Lauraceae), stems and leaf of *Cymbopogon citratus* (D.C) Stapf. (Poaceae), fruits of Citrus medical L. (Rutaceae) as well as honey in ethanol and sucrose experimental model. *BioMedCentral Complementary and Alternative Medecine*. 2014; 14: 507.

23. Radovits T, Korkmaz S, Mátyás C, Oláh A, NémethB, Páli S, Hirschberg K, Zubarevich A, Gwanmesia P, Li S, Loganathan S, Barnucz E, Merkely B, Szabó G. An altered pattern of myocardial histopathological and molecular changes underlies the different characteristics of type-1 and Type-2 diabetic cardiac dysfunction. *Journal of Diabetes Research*. 2015 ; 12 : 4-10.

24. Roy J, Prince M. Preventive effects of p-coumaric acid on cardiac hypertrophy and alterations in electrocardiogram, lipids, and lipoproteins in experimentally induced myocardial infarcted rats. *Food and chemical toxicology*, 2013 ; 348-354.

25. Kela A, Reddy L, Thombre P. E.C.G. findings in normal rats and after administration of isoproterenol. *Indian. Journal of Physiology and. Pharmacology*. 1980 ; 24 :84-90.

26. Thippeswamy B, Thakker S, Tubachi S, Kalyani A, Netra M, Patil U, Desai S, Gavimath C, Veerapur V. Cardioprotective Effect of Cucumis trigonus Roxb on Isoproterenol-Induced Myocardial Infarction in Rat. *American Journal of Pharmacology and Toxicology*. 2009 ; 4 :29-37.

27. Yamaji T, Fukuhara T, Kinoshita M. Increased capillary permeability to albumin in diabetic rat myocardium. Circ Res. 1993 ;72 : 947–957.

28. Abbas M, Sakr H. Simvastatin and vitamin E effects on cardiac and hepatic oxidative stress in rats fed on high fat diet. *Journal of physiology and hiochemestry*. 2013 ; DOI 10.1007/s13105-013-0250-y.

29. Queenthy S, Babu J. Diosmin exhibits anti-hyperlipidemic effects in isoproterenol induced myocardial infarcted rats. *European Journal of Pharmacology*. 2013 ; 718 : 213-18.

30. Chait A, Eckel R. Lipids. lipoproteins and cardiovascular disease: clinical pharmacology now and in the future. *The Journal of Clinical Endocrinology & Metabolism*. 2016 ; 101 : 804-814.

31. Hussain K, Ansari A, Ferder L. Alcohol-induced hypertension. Mechanism and prevention. *World Journal of Cardiology*. 2014; 6:245-252.

32. Maruhashi T, Higashi Y. Pathophysiological Association between Diabetes Mellitus and Endothelial Dysfunction. *Antioxydants*. 2021 ; 13 : 4-10.

33. Erukainure O, Msomi N, Beseni B, Salau F, Ijomone O, Koorbanally N, Islam M. *Cola nitida* infusion modulates cardiometabolic activities linked to cardiomyopathy in diabetic rats. *Food and chemical toxicology*. 2021 ; 12 : 2-10.

34. Shalaby M, Abd El Latif H, El Yamani M, Galal M, Kamal S, Sindi I, Masaood R. Therapeutic activity of sarpogrelate and dopamine D2 receptor agonists on cardiovascular and renal systems in rats with alloxan-induced diabetes. *BMC Pharmacology and Toxicology*. 2021 ; 9 : 2-8.

35. Ozaki K, Terayama Y, Matsuura T, Narama I. Effect of combined dyslipidemia and hyperglycemia on diabetic peripheral neuropathy in alloxan-induced diabetic WBN/ Kob rats. *Journal of Toxicologic Pathology*. 2018 ; 31 : 125-133.

36. Babu P, Sabitha K, Shyamaladevi C. Green tea impedes dyslipidemia. lipid peroxidation. protein glycation and ameliorates Ca^{2+}-ATPase and Na^{+}/K^{+}-ATPase activity in the heart of streptozotocin-diabetic rats. *Chemico-Biological Interactions*. 2006 ; 162 : 157-164.

37. Meeran M, Selvaraj G. Thymol attenuates altered lipid metabolism in β-adrenergic agonist induced myocardial infarcted rats by inhibiting tachycardia, altered electrocardiogram, apoptosis and cardiac hypertrophy. *Journal of functional foods*. 2015 ; 14 : 51-61.