Scientific Research Publishing

Journal of Biosciences and Medicines, 2023, 11, 277-302
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Acute and Prolonged Effects of a Polyherbal Formulation on Blood Glucose, Lipid Profile and Liver Function in Normal and Streptozotocin-Induced Diabetic Rats

Marie Claire Tchamadeu[1*], Hélène Elena Ndame[1], Calvin Zangueu Bogning[1], Modeste Wankeu-Nya[1], Patience Emambo[1], Olga Sol Fonga[1], Christian Takoukam Tenezogang[1], Alain Bertrand Dongmo[1], Siméon Pierre Choukem[2]

[1]Department of Biology and Physiology of Animal Organisms, Faculty of Science, University of Douala, Douala, Cameroon
[2]Faculty of Medicine and Pharmaceutical Sciences, University of Dschang, Dschang, Cameroon
Email: *marieclaire_tchamadeu@yahoo.fr

How to cite this paper: Tchamadeu, M.C., Ndame, H.E., Bogning, C.Z., Wankeu-Nya, M., Emambo, P., Fonga, O.S., Tenezogang, C.T., Dongmo, A.B. and Choukem, S.P. (2023) Acute and Prolonged Effects of a Polyherbal Formulation on Blood Glucose, Lipid Profile and Liver Function in Normal and Streptozotocin-Induced Diabetic Rats. *Journal of Biosciences and Medicines*, **11**, 277-302.
https://doi.org/10.4236/jbm.2023.1111024

Received: September 25, 2023
Accepted: November 21, 2023
Published: November 24, 2023

Abstract

Background: Few studies have often focused on medicinal plant mixtures, yet the most used in low-and middle-income areas as alternative drug to treat diseases. **Objective:** To evaluate the antidiabetic effects of *Emilia coccinea* (Ec) (*Asteraceae*), *Scoparia dulcis* (Sd) (*Plantaginaceae*) and *Tetracarpidium conophorum* (Tc) (*Euphorbiaceae*) aqueous extracts mixture (EcSdTc) in rats. **Methodology:** Single plant aqueous extracts (Ec, Sd and Tc) and their mixtures (EcSd, EcTc, SdTc and EcSdTc) (each at the doses of 125 and 250 mg/kg body weight respectively) were evaluated in acute administration on blood glucose in normal, glucose-overloaded and diabetic rats; then EcSdTc mixture was assessed in prolonged administration (21 days) on blood glucose, body weight, serum biochemical and antioxidant parameters in diabetic rats. Diabetes was induced by single intraperitoneal administration of streptozotocin (STZ; 50 mg/kg), and glibenclamide (10 mg/kg) was used as standard drug. **Results:** In acute administration, EcTc250, EcSdTc125, SdTc250, SdTc125, EcSd250, and EcSdTc250 extracts mixtures reduced ($p < 0.05$ - $p < 0.01$) the blood glucose of normal rats by 21.89%, 19.04%, 17.94%, 17.69%, 17.28%, and 15.07% while EcTc250, Tc125, Tc250, Ec250, EcSdTc250 and Sd250 extracts reduced ($p < 0.0001$ - $p < 0.05$) the glycemia of glucose-fed rats by 22.43%, 19.62%, 17.60%, 13.26%, 12.58%, and 11.77% respectively, compared to their respective Normal and Hyperglycemic controls. Prolonged administration of the EcSdTc mixture (125 and 250 mg/kg) in diabetic rats decreased ($p < 0.05$ - $p < 0.001$) the blood glucose, serum triglycerides, total cholesterol, LDL-cholesterol, atherogenic index, serum ALAT/ASAT activities and liver MDA,

increasing ($p < 0.05$ - $p < 0.001$) the body weight, HDL-cholesterol, total proteinemia, and liver SOD, Catalase, NO, GSH activities or levels, comparatively to diabetic control. **Conclusion:** EcSdTc aqueous extracts mixture has potent hypoglycemic and antidiabetic effects, probably due to their bioactive compounds synergistic and/or additive actions, justifying its traditional use as alternative remedies.

Keywords

Emilia coccinea, *Scoparia dulcis*, *Tetracarpidium conophorum*, Traditional Medicinal Potion, Diabetes Mellitus, Streptozotocin

1. Introduction

The worldwide occurrence of the COVID-19 pandemic has reinforced the evidence that diabetes and its long-term complications are risk factors for morbidity and mortality in case of additional conditions. Indeed, studies adjusted for age, gender and other risk factors have found that lower blood glucose control as measured by higher HbA1c, was associated with more unfavorable COVID-19 cases leading to hospitalization, admission to intensive care and death [1]. Therefore, improving first of all national policies and programs for blood glucose management and control in populations with diabetes is necessary and essential to better manage and reduce subsequent illnesses and their consequences. Diabetes mellitus is a metabolic disorder characterized by an increase in the blood glucose level linked to carbohydrate, lipid and protein metabolism alterations [2]. Diabetes mellitus is one of the most common diseases of the 21st century [3], resulting from insufficient insulin secretion by pancreatic beta cells and/or resistance of target tissues to the insulin action [4]. The global number of people with diabetes continuously increased; The estimations of 463 million worldwide, 19 million in Africa and 615 thousand cases in Cameroon in 2019 have increased to 537 million, 24 million and 620.8 thousand respectively in 2021 [1] [5]. The prevalence, undiagnosed cases, deaths from diabetes and increasing spending are and will be even more alarming in poor and middle-income regions. Projections predict that 783 million people worldwide and 55 million in Africa would be affected by 2045 [1]. Type 1 diabetes, one of the most common forms is very common in children and adolescents and results from autoimmune destruction of the insulin-producing pancreatic beta cells. People with type 1 diabetes may improve their living conditions through adequate daily insulin therapy, blood sugar monitoring, adoption of healthy diet and lifestyle, and physical activity practice [6]. However, access to fundamental components of care for people living with diabetes as self-treatment tools, insulin and self-management education is limited or absent, even very expensive for low- and middle-income families, which motivates them to use medicinal plants as alternatives [7]. This situation, which has been strongly observed recently in the case of COVID-19, has rein-

forced in many low- and middle-income countries, and even developed countries, the scientific debate around the need for and the interest of traditional or empirical medicinal knowledge in the management of pathologies. Many empirical used medicinal plants whose chemical compositions and pharmacological properties are often unknown can be in individual preparation, but are most often in combination as polyherbal formulations or potions. Various plants combinations can give synergistic, antagonistic and indifferent possible interactions between particular compounds, which is important in development of food products [8] and probably traditional polyherbal drugs. Such a traditional therapeutic potion made from mixture of *Emilia coccinea* (Ec), *Scoparia dulcis* (Sd) and *Tetracarpidium conophorum* (Tc), (EcSdTc) is used in Cameroon coastal region to treat mostly malaria, but also cardiovascular pathologies. Indeed, the hypoglycemic and antidiabetic properties of each of these three plant species have been already highlighted [9]-[19]. However, the assessment of therapeutic potential of their mixture (EcSdTc) used in Cameroonian pharmacopoeia has not yet been the subject of a prior study. In order to validate the empirical use of EcSdTc and the hypothesis according to which "the mixture of potentially antidiabetic plants (*E. coccinea*, *S. dulcis* and *T. conophorum*) would possess therapeutic activities, even better than each of its individual plants", it has being therefore proposed to evaluate the effects of EcSdTc plants aqueous extracts mixture on streptozotocin-induced diabetes mellitus in rat. The hypoglycemic and antihyperglycemic effects of each plant extract and some of their mixtures were first assessed in normal rat.

2. Materials and Methods

2.1. Ethical Approval

The study was conducted in respect of all Guidelines for Care and Use of Laboratory Animals as described in the European Community Guidelines (EEC Directive 2010/63/EU of the September 22, 2010), and after obtaining approval for Animal Experimentation No. 2430/CEI-UDO/08/2020/M from the institutional ethics committee for human health research of the University of Douala.

2.2. Chemicals

Streptozotocin (STZ) was purchased from Sigma Chemical Co. (Saint Louis, MO, USA), Glibenclamide (GB) obtained from Mylan Laboratory, Accu-chek Plus blood glucose test strips and glucometer from Roche Diagnostics (Mannheim, Germany), and all other reagents and chemicals (Extra pure analytical grade) from common commercial suppliers were used in the study.

2.3. Plant Materials

Fresh whole plants of *Emilia coccinea*, *Scoparia dulcis* and liana of *Tetracarpidium conophorum* were collected in Makoma village (Littoral region, Cameroon) after the botanical identification at the Cameroon National Herbarium of

Yaoundé in comparison to the respective voucher specimens: No. 42027/HNC, No. 702/HNC and No. 15425/SQF Cam respectively.

2.4. Preparation of Extracts

The different collected plants parts were separately dried at room temperature and crushed into powder using a Moulinex. Each dried plant powder (200 g) was infused in 1500 ml of distilled water for 24 h, then filtered using No. 3 Wattman paper. Each filtrate was evaporated in the oven at 40˚C yielding 23.15 g, 12.34 g and 14.80 g (W/W 11.58%, 06.17% and 07.40%) of crude dried extract respectively for *Emilia coccinea*, *Scoparia dulcis* and *Tetracarpidium conophorum* and stored at −20˚C until use.

The choice of doses to be studied was first made and based on the traditional human dose (39.87 mg/kg) determined by following the traditional instructions for preparing the plants mixture EcSdTc. Then the human dose was translated to the rat dose as:

$$\text{Rat dose (mg/kg)} = (\text{Human dose (mg/kg)}) * k \text{ (with } k = 6.2) \text{ [20].}$$

The obtained rat dose adjusted to the fixed upper/lower value and its half were used for the study.

For administration to rats in each experiment, plants aqueous extract concentrated solutions were prepared by dissolving weighed quantities of crude dried extract of each plant or their combination in distilled water to obtain 12.5 mg/ml stock solutions every 3 days according to Table 1.

2.5. Animals

Male albino Wistar rats aged 2.5 to 3 months weighing 200 to 250 g were used. They were reared in the animal facility of the Faculty of Science, University of Douala and housed in cages (5 animals per cage). Rats had free access to tap water and standard food, under ambient temperature and natural lighting (alternating day/night).

Table 1. Preparation of the different extract solutions.

Extract type	Plants names	Abréviations	Preparation
Individual PE (w)	*Emilia coccinea*	Ec	375 mg of extract + 30 mL of DW
	Scoparia dulcis	Sd	375 mg of extract + 30 mL of DW
	Tetracarpidium conophorum	Tc	375 mg of extract + 30 mL of DW
Two PEM (w/w)	*E. coccinea* + *S. dulcis*	EcSd	Ec (187.5 mg) + Sd (187.5 mg) + DW (30 mL)
	E. coccinea + *T. conophorum*	EcTc	Ec (187.5 mg) + Tc (187.5 mg) + DW (30 mL)
	S. dulcis + *T. conophorum*	SdTc	Sd (187.5 mg) + Tc (187.5 mg) + DW (30 mL)
	[1/2 * 375 * (extract 1 + extract 2) mg + 30 mL of DW]		
Three PEM (w/w/w)	*E. coccinea* + *S. dulcis* + *T. conophorum*	EcSdTc	Ec (125 mg) + Sd (125 mg) + Tc (125 mg) + DW (30 mL)
	[1/3 * 375 * (Ec + Sd +Tc) mg + 30 mL of DW]		

PE = plant extract; PEM = plants' extracts mixture; w = weight; w/w = weight/weight; w/w/w = weight/weight/weight; DW = distilled water.

2.6. Induction of Diabetes Mellitus

Type 1 diabetes was induced by a single intraperitoneal injection of Streptozotocin (STZ: 50 mg/kg freshly diluted in ice-cold NaCl solution 0.9%) in 12-hour-fasted rats and anesthetized with isoflurane to avoid pain and stress. Normal control rats received by the same route an equal volume of 0.9% NaCl. Seventy-two hours (72 h) after STZ injection, rats with a fasting blood glucose of at least 350 mg/dl were considered diabetic and used in the experiments.

2.7. Measurement of Fasting Blood Glucose

Overnight fasting (12 or 16 hours) blood glucose was determined by glucose-peroxidase method using test strips (Accu-chek Aviva) and an appropriate glucose meter (Accu-chek Aviva Connect, Roche Diagnostics, Germany) as previously described [21] [22] at 0, 1, 2, 3 and 5 h or −30, 0, 30, 60, 90 and 120 minutes for acute experiments, and at 0, 8, 15 and 22 days for sub-chronic experiment.

2.8. Assessment of Acute Effects of Aqueous Extracts of *Emilia coccinea*, *Scoparia dulcis*, *Tetracarpidium conophorum* and Mixtures in Normal Rats

Eighty normoglycemic rats previously subjected to a non-water fast (12 h) were distributed by randomization of the initial blood glucose in 16 groups of 5 rats each as:

Group 1: normal control rats (NC) received distilled water (10 mL/kg).

Group 2: normal rats treated with the glibenclamide (10 mg/kg).

Groups 3-8: normal rats received respectively the aqueous extracts of *Emilia coccinea*, *Scoparia dulcis* and *Tetracarpidium conophorum* (Ec, Sd and Tc respectively) at doses of 125 and 250 mg/kg.

Groups 9-14 received the respective mixtures of two plants extracts (EcSd, EcTc, and SdTc), each at the doses of 125 and 250 mg/kg.

Groups 15 and 16 received the three plants extracts mixture (EcSdTc) at the doses of 125 and 250 mg/kg.

Treatments were administered by gavage; the blood glucose was determined at 0 hour before, and 1, 2, 3 and 5 hours after treatment.

2.9. Assessment of Acute Effects of Aqueous Extracts of *Emilia coccinea*, *Scoparia dulcis*, *Tetracarpidium conophorum* and Their Mixtures in Glucose-Overloaded Normal Rats

Seven days after the previous test (time required for the previous substances administered expiration), the 80 animals above used and 5 others were overnight fasted (16 h non-water fast), then distributed by randomization of the initial glycemia in 17 groups of 5 rats each and treated as follow:

Group 1: normal rats administered with distilled water (10 mL/kg), normal control (NC).

Group 2: normal rats administered with distilled water (10 mL/kg) + D-glucose (5 mg/kg), hyperglycemic control (HGC).

Group 3: normal rats treated with glibenclamide (10 mg/kg) + D-glucose (5 mg/kg).

Groups 4-9: normal rats receiving individual plant extract (Ec, Sd or Tc), each at the doses of 125 and 250 mg/kg + D-glucose (5 mg/kg) respectively.

Groups 10-15: normal rats treated with the respective mixtures of two plants extracts (EcSd, EcTc or SdTc), each at the doses of 125 and 250 mg/kg + D-glucose (5 mg/kg).

Groups 16 and 17: normal rats treated with the mixture of the three plants extracts (EcSdTc) at doses of 125 and 250 mg/kg + D-glucose (5 mg/kg).

The water, glibenclamide and plants extracts doses were administered by gavage, immediately after the first blood glucose monitoring and groups formation (−30 min). Thirty minutes after, a second blood glucose monitoring (0 min) was immediately followed by a D-glucose (5 g/kg) oral administration to rats of groups 2 to 17. The blood glucose was again determined at 30, 60 and 120 minutes after D-glucose.

2.10. Assessment of Acute and Prolonged Administration of the Mixture of Aqueous Extracts of *Emilia coccinea*, *Scoparia dulcis* and *Tetracarpidium conophorum* in Diabetic Rats

After diabetes screening, animals were first subjected to a hypoglycemic test in acute treatment for 5 h as previously described with normoglycemic rats before being subjected to the prolonged treatment at the following days. Briefly, twenty (20) confirmed diabetic and 5 normoglycemic 12-hour fasted rats (prior to water) were divided into 5 groups of 5 rats each according to the initial glycemia (at 0 h) as:

- A healthy normal control (NC) group consisting of normoglycemic rats receiving distilled water (10 mL/kg).
- A diabetic control (DC) group consisting of diabetic rats receiving distilled water (10 mL/kg).
- A glibenclamide diabetic group (GBD) containing diabetic rats treated with glibenclamide (10 mg/kg).
- and two groups of diabetic rats treated with the mixture of the three plants extracts (EcSdTc) at respective doses of 125 and 250 mg/kg of body weight.

The animals subsequently received by gavage single administration of the various treatments as described above, and the blood sugar was again measured at 1, 2, 3 and 5 hours after, in order to evaluate the acute effects of the mixture in diabetic rats. Then, the animals continued to receive the different treatments daily for 21 days during which blood glucose was measured weekly (at days 7, 14 and 21), body mass, water and food intakes daily, and the serum and liver biochemical parameters at the end of treatment.

2.11. Sacrifice, Blood Collection and Serum Biochemical Analysis

At the 22nd day, the 12-hour overnight-fasted animals were anesthetized and sacrificed by decapitation. Arteriovenous blood was collected in dry tubes and

centrifuged at 3000 rpm for 15 minutes. The serum obtained was stored in Eppendorf tubes at −20˚C for serum biochemical parameters determination, using commercial diagnostic kits (SGM ITALIA, Rome, ITALY) for total proteins (Biuret), creatinine (colorimetric), Triglycerides (GPO-PAD method), total cholesterol (CHOD-PAD Method), HDL-cholesterol (colorimetric), ALT (colorimetric), AST (colorimetric). Serum LDL-cholesterol [23] and atherogenic risk index (ARI) [24] were calculated.

2.12. Liver Oxidative Stress Markers Analysis

A part of each liver immediately removed after the sacrifice was crushed, homogenized in Tris-HCl buffer and centrifuged at 3000 rpm for 15 minutes. The supernatant obtained was stored at −20˚C for SOD, catalase (activities), reduced glutathione, MDA and NO (levels) determination in liver.

2.13. Statistical Analysis of Data

All data were expressed as the mean ± Standard Error of the Mean (ESM). Statistical analysis of data was done using GraphPad Prism 5.03 software. Analysis of variance tests "one-way ANOVA" followed by Bonferroni post-test and "two-way ANOVA" followed by Tukey post-test were used. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Acute Effects of *Emilia coccinea*, *Scoparia dulcis* and *Tetracarpidium conophorum* Aqueous Extracts and Their Mixtures in Normal Rats

The plants (*Emilia coccinea*, *Scoparia dulcis* and *Tetracarpidium conophorum*) aqueous extracts administered individually did not reduced significantly the blood glucose of normal rats (**Figure 1(A)**). But, their mixtures have more or less decreased the blood glucose of normal rats from the 1rst hour compared to the normal control (EcTc250, SdTc125 and EcSdTc125; $p < 0.05$) and from the 2nd hour compared to the initial blood glucose [(EcSd250, $p < 0.05$); (EcTc250, $p < 0.01$); (SdTc250, $p < 0.001$) and (EcSdTc125; $p < 0.01$)], until the fifth hour following the administration where the EcTc mixture (250 mg/kg) induced the highest decrease compared to T0 (38.85%, $p < 0.001$) and normal control (33.10%, $p < 0.001$) (**Figure 1(B)** and **Figure 1(C)**). The major effects of EcSdTc mixture on normal blood glucose were observed at its low dose (125 mg/kg) at the third hour compared to normal control (36.41%, $p < 0.01$) and T0 glycemia (32.25 %, $p < 0.001$) (**Figure 1(C)**). Glibenclamide better decreased the glycemia of normal rats than the various plant extracts, from the 1st to the 5th hour, where its effects were maximal compared to NC (56.8%; $p < 0.001$) and T0 (61.7%; $p < 0.001$) (**Figures 1(A)-(C)**).

In sum, the calculated area under the glycaemia curve (AUC) indicating the global effect in each group showed seven effective hypoglycemic treatments in the following decreasing order: glibenclamide (10 mg/kg) (32.48%; $p < 0.0001$), fol-

lowed by the mixtures EcTc250 (21.89%; $p < 0.01$), EcSdTc125 (19.04%; $p < 0.01$), SdTc250 (17.94%; $p < 0.05$), SdTc125 (17.69%; $p < 0.05$), EcSd250 (17.28%; $p < 0.05$), and EcSdTc250 (15.07%; $p < 0.05$) at indicated doses (Figure 1(D)).

3.2. Antihyperglycemic Effects of *Emilia coccinea*, *Scoparia dulcis*, *Tetracarpidium conophorum* Aqueous Extracts and Their Mixtures in Glucose-Overloaded Normal Rats

Thirty minutes after treatments administration (T0), the blood glucose did not vary between the groups, nor compared to the initial glycaemia (at T-30) in each group (Figures 2(A)-(C)). However, from the 30th to the 120th minutes following the glucose administration, the mean glycaemia at each given time changed significantly ($p < 0.05$ - $p < 0.001$) in all glucose-fed rats groups compared to T0 glycaemia and the NC (Figures 2(A)-(C)).

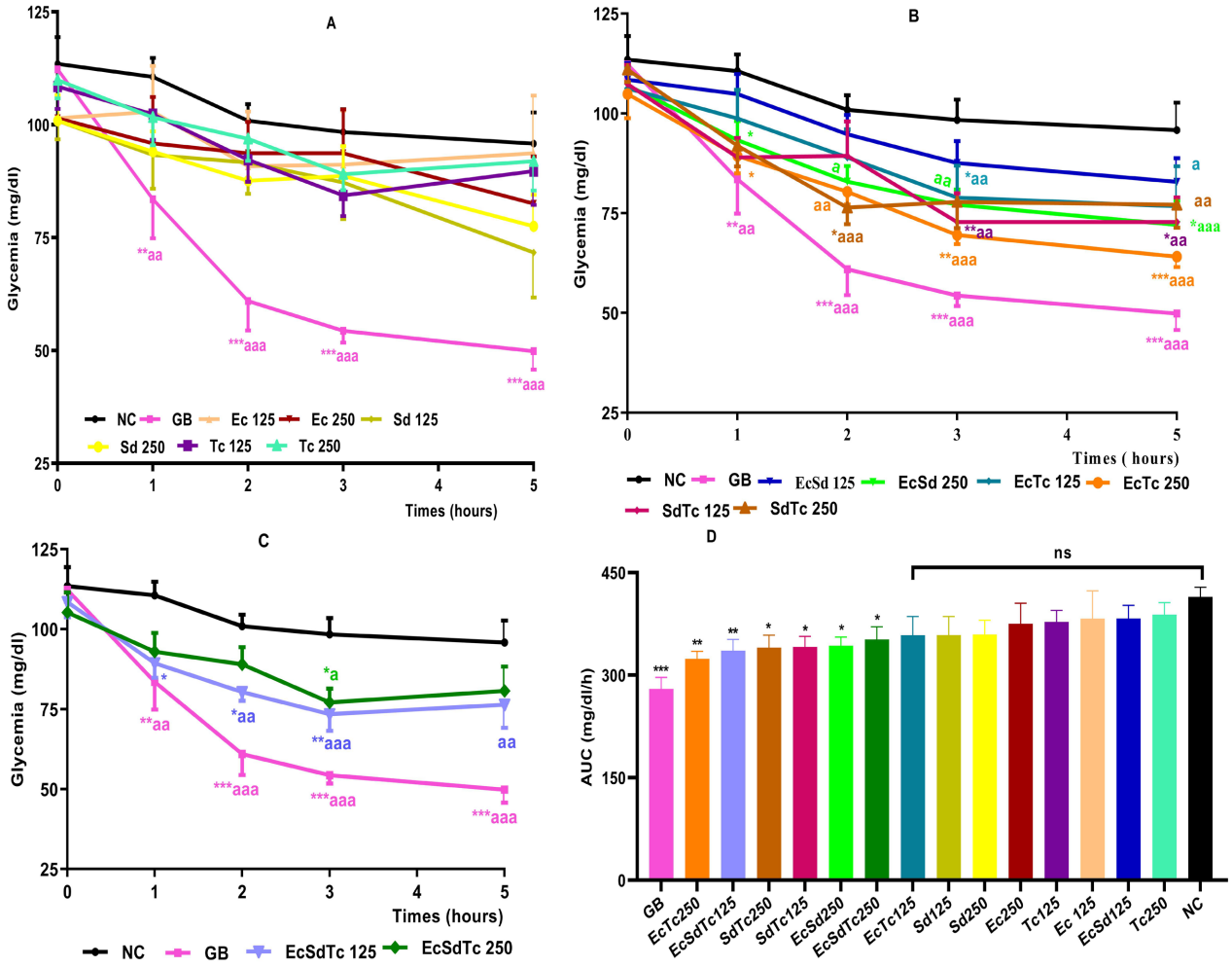

Figure 1. Blood glucose variation in normal rats receiving individual (*E. coccinea*, *S. dulcis* and *T. conophorum*) (A), and mixtures of two (B) or three (C) aqueous plants extracts, and estimated AUC (D). Each point represents the Mean ± ESM; n = 5; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ vs normal control; $^{a}p < 0.05$; $^{aa}p < 0.01$; $^{aaa}p < 0.001$ vs baseline glycemia (0 h). NC = Normal Control; GB = glibenclamide; Ec = *E. coccinea*; Sd = *S. dulcis*; Tc = *T. conophorum*. EcSd, EcTc, SdTc and EcSdTc = respectively *E. coccinea*-*S. dulcis*, *E. coccinea*-*T. conophorum*, *S. dulcis*-*T. conophorum*, and *E. coccinea*-*S. dulcis*-*T. conophorum* extracts mixtures, at the indicated doses; AUC = area under the curve.

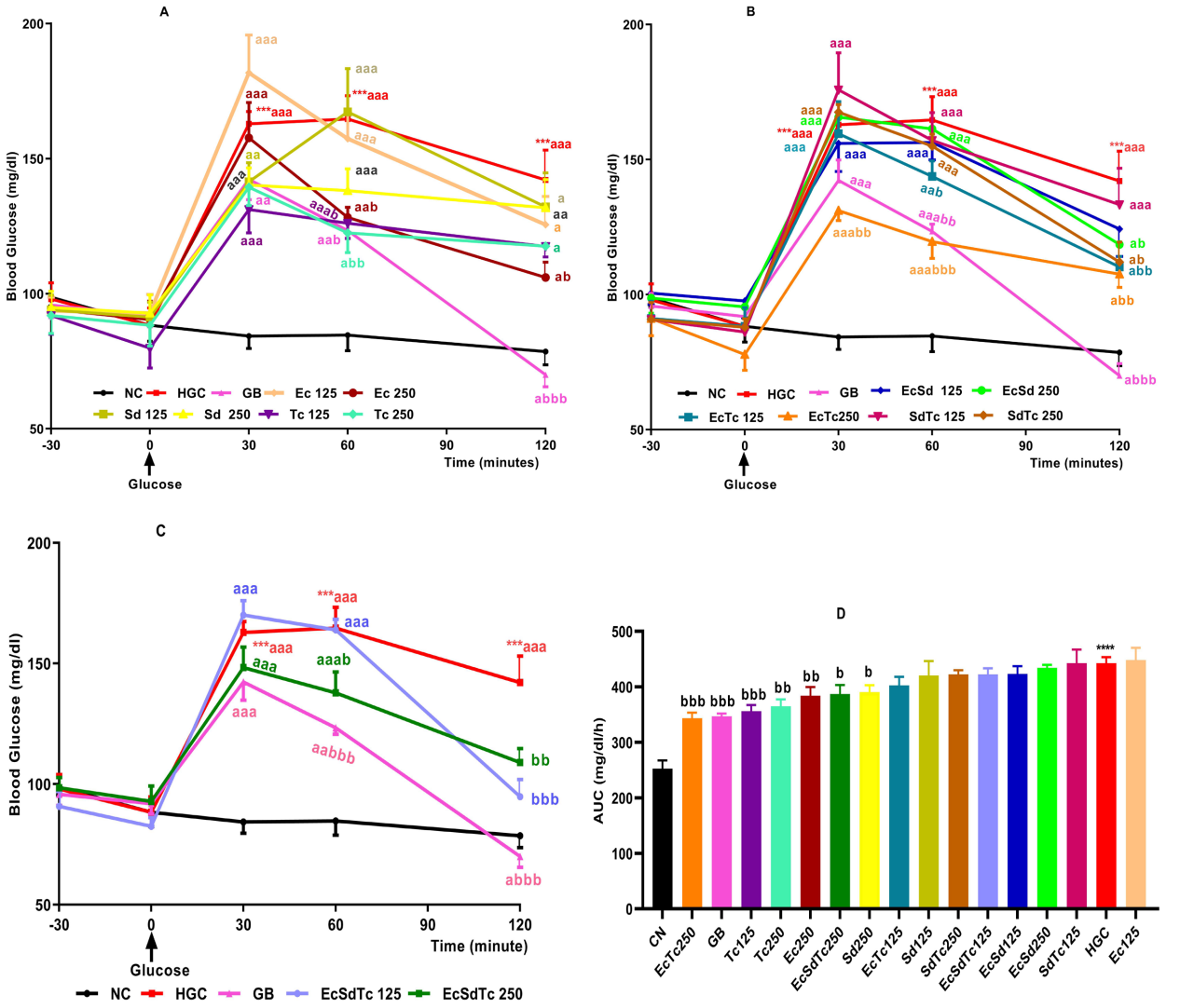

Figure 2. Single doses effects of *E. coccinea*, *S. dulcis* and *T. conophorum* aqueous extracts (A) and their mixtures by two (B) or three (C) on oral glucose tolerance, and estimated AUC (D) in normal rat. Each point represents the Mean ± ESM; n = 5; ***p < 0.001 vs NC; $^{a}p < 0.05$; $^{aa}p < 0.01$; $^{aaa}p < 0.001$ vs T_0; $^{b}p < 0.05$; $^{bb}p < 0.01$; $^{bbb}p < 0.001$ vs HGC; NC = Normal Control; HGC = Hyperglycemic control; GB = Glibenclamide; Ec = *E. coccinea*; Sd = *S. dulcis*; Tc = *T. conophorum*. EcSd, EcTc, SdTc and EcSdTc = respectively *E. coccinea*-*S. dulcis*, *E. coccinea*-*T. conophorum*, *S. dulcis*-*T. conophorum*, and *E. coccinea*-*S. dulcis*-*T. conophorum* extracts mixtures, at the indicated doses; AUC = area under the curve.

Overall, the calculated area under the blood glucose curve (AUC), summarizing the mean blood glucose level changes from T0 to T120 in each group indicates a significant blood glucose level increase in HGC rats (67.44 %; $p < 0.0001$) compared to NC (Figure 2(D)). The individual extracts (Ec250, Sd250, Tc125 and Tc250), mixtures (EcTc250 and EcSdTc250) and glibenclamide (10 mg/kg) at indicated doses, significantly and efficiently reduced the hyperglycaemia in the following decreasing order: EcTc250 (22.43%; $p < 0.0001$), glibenclamide (21.71%; $p < 0.0001$), Tc125 (19.62%; $p < 0.001$), Tc250 (17.60%; $p < 0.01$), Ec250 (13.26%; $p < 0.01$), EcSdTc250 (12.58%; $p < 0.05$) and Sd250 (11.77%; $p < 0.05$), all compared to HGC (Figure 2(D)).

3.3. Acute and Prolonged Effects of *Emilia coccinea*, *Scoparia dulcis* and *Tetracarpidium conophorum* Aqueous Extracts Mixture (EcSdTc) on Blood Glucose Levels in Diabetic Rats

Seventy-two hours (72 h) after streptozotocin (STZ) administration, the normal glycaemia increased nearly 3.36 times ($p < 0.001$) (diabetic control (DC) compared to normal control (NC) on day 0 (D0)).

Then, acute administration of the EcSdTc plants extracts mixture (125 and 250 mg/kg) and glibenclamide did not reduced the hyperglycemia of diabetic rats during the 5 hours following drugs administration compared to DC rats. But, compared to the initial glycaemia (0 h), the EcSdTc mixture at the dose of 250 mg/kg significantly reduced the hyperglycaemia by 26.17% ($p < 0.01$) at the 5th hour (Figure 3(A)).

However, in prolonged treatment, the hyperglycaemia remained elevated in DC rats throughout the 21 days of treatment compared to NC (Figure 3(B)). At the 21st day of treatment, the plant extracts mixture (EcSdTc)-treated diabetic rats showed significant and maximal blood sugar drops of 49.70% ($p < 0.001$) and 56.75% ($p < 0.001$) at the doses of 125 and 250 mg/kg respectively while the glibenclamide decreased the blood glucose in diabetic rats by 47.86% ($p < 0.001$), all compared to diabetic control (DC). Moreover, the 21st-day blood glucose decreases were by 44.60% ($p < 0.05$), 56.11% ($p < 0.01$) and 45.91% ($p < 0.01$) respectively for EcSdTc125, EcSdTc250 and glibenclamide (10 mg/kg) compared to D0 blood glucose in each group (Figure 3(B)).

3.4. Prolonged Effects of EcSdTc Mixture on Weight Gain, Serum Total Protein and Creatinine in Diabetic Rats

During the 21 days of treatment, normal control rats (NC) showed progressive and significant weight gain, with a maximal of 62.73% ($p < 0.001$) at D21 compared to D0. Compared to NC group, the diabetic control rats (DC) showed

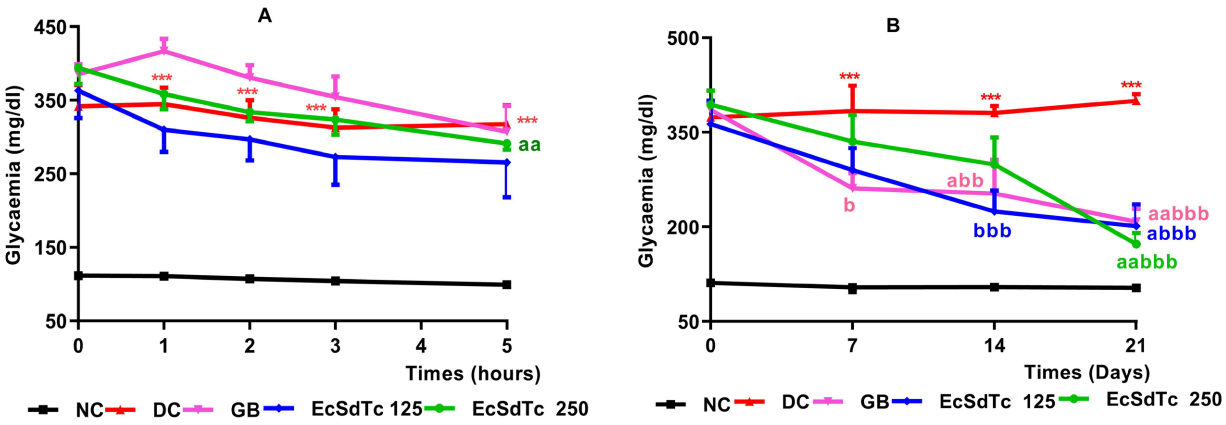

Figure 3. Acute (A) and prolonged (B) effects of the EcSdTc aqueous plant extracts mixture on blood glucose levels in STZ-diabetic rats. Each point represents the mean ± ESM; n = 5; ***$p < 0.001$ vs NC; $^{a}p < 0.05$; $^{aa}p < 0.01$; $^{b}p < 0.05$; $^{bb}p < 0.01$; $^{bbb}p < 0.001$ vs DC; NC = Normal Control; DC = diabetic control; GB = Glibenclamide; EcSdTc = mixture of aqueous extracts of *E. coccinea*, *S. dulcis* and *T. conophorum* at the indicated doses.

progressive and significant ($p < 0.001$) body weight loss of 20.10%, 37.25% and 44.86% respectively at D7, D14 and D21 of treatment (**Figure 4(A)**), associated with significant serum total protein decrease (35.72%; $p < 0.05$) (**Figure 4(B)**) and serum creatinine increase (98.76%; $p < 0.001$) (**Figure 4(C)**) at D21. The administrated EcSdTc aqueous extracts mixture induced at D21 of treatment, significant body weight gain of 25.99% ($p < 0.01$) and 38.28% ($p < 0.001$), significant proteinemia increase of 53.34% ($p < 0.001$) and 53.12% ($p < 0.001$), each respectively at the doses of 125 and 250 mg/kg, all compared to DC group. The elevated creatininemia was decreased only by the dose extract of 250 mg/kg (55.91%; $p < 0.05$). The glibenclamide (10 mg/kg) also increased significantly the proteinemia (45.53%; $p < 0.01$), but without any significant effects on serum creatinine and body weight loss in diabetic rats compared to DC group (**Figures 4(A)-(C)**).

3.5. Prolonged Effects of the EcSdTc Aqueous Extracts Mixture on Lipid Profile

The lipid profile of diabetic control rats (DC) showed significant ($p < 0.001$) increase levels in serum triglycerides (59.67%), total cholesterol (154.30%) and LDL-cholesterol (374.58%), decreased serum HDL-cholesterol (56%) and high atherogenic risk index (normal ARI elevated by 6 times), compared to normal control rats (NC) (**Table 2**).

The administration of the EcSdTc extracts mixture significantly ($p < 0.001$) decreased serum triglycerides, total cholesterol and LDL-cholesterol levels, and increased HDL-cholesterol by 42.95%, 43.58%, 65.27% and 101.17% respectively at the dose of 125 mg/kg, and by 30.38%, 24.39%, 49.52% and 74.13% respectively at the dose of 250 mg/kg, all compared to DC; Thus the observed DC rats atherogenic risk index was reduced by 66.28% and 55.92% respectively at 125 and 250 mg/kg EcSdTc doses. Glibenclamide (10 mg/kg) reduced significantly ($p < 0.001$) the serum triglycerides, total cholesterol and LDL-cholesterol levels while increasing serum HDL-cholesterol levels respectively by 38.17%, 43.58%, 73.87% and 67.69%, with an atherogenic risk decreased by 66.78% compared to DC rats (**Table 2**).

Table 2. Lipid profile changes in aqueous extracts mixture (EcSdTc)-treated diabetic rats.

Parameters	Treatments				
	NC	DC	GB	EcSdTc 125	EcSdTc 250
Trigly. (mg/dl)	217.11 ± 4.93	346.66 ± 1.63***	214.33 ± 14.04^{bbb}	197.77 ± 1.89^{bbb}	241.33 ± 5.74^{bbb}
Total Chol. (mg/dl)	113.65.29	279.80 ± 3.92***	193 ± 20.87^{bbb}	189.42 ± 80^{bbb}	211.53 ± 5.85^{bb}
HDL-Chol. (mg/dl)	104.83 ± 1.68	46.12 ± 0.83***	77.34 ± 1.83^{bbb}	92.78 ± 3.71^{bbb}	80.30 ± 4.47^{bbb}
LDL-Chol. (mg/dl)	34.63 ± 5.28	164.35 ± 5.08***	42.94 ± 1630^{bbb}	57.07 ± 7.41^{bbb}	82.96 ± 8.22^{bbb}
ARI	1.08 ± 0.05	6.08 ± 0.19***	2.02 ± 0.23^{bbb}	2.05 ± 0.08^{bbb}	2.68 ± 0.16^{bbb}

Each value represents mean ± ESM; n = 5; ***$p < 0.001$ vs. healthy control; $^{bb}p < 0.01$; $^{bbb}p < 0.001$ vs. diabetic control; NC = Normal control; DC = Diabetic Control; GB = glibenclamide; EcSdTc = *Emilia coccinea*, *Scoparia dulcis* and *Tetracarpidium conophorum* aqueous extracts mixture at indicated doses.

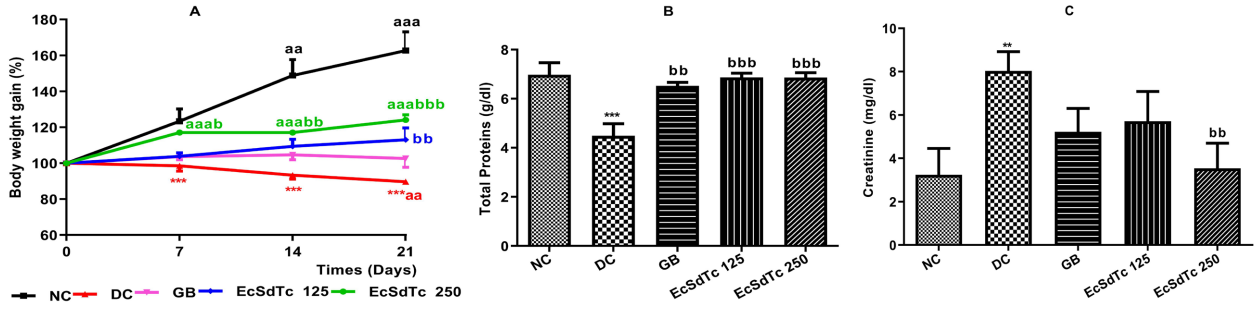

Figure 4. Weight gain (A), proteinemia (B) and creatinemia (C) changes in aqueous extracts mixture (EcSdTc)-treated diabetic rats. The points represent the mean ± ESM; n = 5; **p < 0.01; ***p < 0.001 vs healthy control; ^{aa}p < 0.01; ^{aaa}p < 0.001 vs initial weight; ^{b}p < 0.05; ^{bb}p < 0.01; ^{bbb}p < 0.001 vs diabetic control. NC = Normal Control; DC = Diabetic control; GB = Glibenclamide; EcSdTc = mixture of aqueous extracts of *Emilia coccinea*, *Scoparia dulcis* and *Tetracarpidium conophorum* at the doses indicated.

3.6. Prolonged Effects of the EcSdTc Aqueous Extracts Mixture on Liver Function

3.6.1. Effects on Serum ALAT/ASAT Activities

Streptozotocin administration significantly (p < 0.001) increased by 5.82 and 6.04 times respectively the normal ALAT and ASAT activities in diabetic rats (DC rats compared to NC rats) (**Figure 5(A)**, **Figure 5(B)**). The EcSdTc extracts mixture at the doses of 125 and 250 mg/kg induced significant decreases (p < 0.001) in ALAT (87.92% and 74.05% respectively) (**Figure 5(A)**) and ASAT (82.82% and 91.71% respectively) activities (**Figure 5(B)**), while glibenclamide only decreased ASAT activity (63.42%; p < 0.01) (**Figure 5(B)**), all Compared to diabetic control rats.

3.6.2. Effects on Some Liver Oxidative Stress Parameters

Untreated diabetic rats (DC) showed increased MDA level (71.01%; p < 0.01), as well as decreased GSH level (66.67%; p < 0.001), NO (70.65%; p < 0.01) level and SOD (93.41%; p < 0.001) and Catalase (75.40%; p < 0.001) activities in liver, compared to healthy rats (NC) (**Table 3**). The EcSdTc mixture at the dose of 125 mg/kg administrated to diabetic rats significantly (p < 0.001) decreased the MDA (47.45%), increased the GSH (163.81%), and normalized the NO level and SOD activity without enhancing significantly the catalase activity. The high dose (250 mg/kg) decreased the MDA (55.08%; p < 0.001) level, and increased the GSH (101.32%; p < 0.05) and NO (p < 0.05) levels and the SOD (p < 0.001) and Catalase (p < 0.01) activities in diabetic treated rat's liver compared to DC rats. Glibenclamide also reduced the MDA (47.4%; p < 0.001) and NO (p < 0.01) levels, and increased the SOD (p < 0.05) and catalase (p < 0.001) activities without any significant effect on GSH in liver, compared to DC rats (**Table 3**).

4. Discussion

Although lifestyle changes (diet, exercise, weight loss) and conventional medications showed moderate beneficial effects, the incidence and burdens of diabetes continue increasing, requiring recourse to other therapeutic means such as phytotherapy. Chronic patients sometimes use plants alone or in combination, even

Table 3. Malondialdehyde (MDA), reduced Glutathione (GSH) and NO levels, and SOD and CAT activities in liver of aqueous extracts mixture (EcSdTc)-treated diabetic rats.

Antioxidant Parameters	Treatments				
	NC	DC	GB	EcSdTc 125	EcSdTc 250
MDA (µmol/g)	0.69 ± 0.13	1.18 ± 0.07**	0.53 ± 0.02^{bbb}	0.62 ± 0.04^{bbb}	0.58 ± 0.05^{bbb}
GSH (µmol/g)	4.6 ± 0.54	1.51 ± 0.11***	2.7 ± 0.22	4.00 ± 0.12^{bbb}	3.04 ± 0.11^{b}
SOD (U/min/g)	5699.84 ± 908.21	434.51 ± 45.26***	3181.17 ± 168.82^{b}	3732.62 ± 155.52^{bb}	4980.57 ± 538.92^{bbb}
CAT (mmol H_2O_2/min/g)	31.96 ± 3.42	7.86 ± 0.41***	23.82 ± 3.01^{bbb}	11.26 ± 1.32	22.14 ± 1.30^{bb}
NO (µmol/g)	5.35 ± 0.86	1.57 ± 0.02**	5.30 ± 0.73^{bb}	6.47 ± 0.49^{bbb}	4.41 ± 0.24^{b}

Each value represents the mean ± ESM; n = 5; **p < 0.01; ***p < 0.001 vs NC; ^{b}p < 0.05; ^{bb}p < 0.01; ^{bbb}p < 0.001 vs DC; NC = normal control; DC = diabetic control; GB = glibenclamide; EcSdTc = *Emilia coccinea*, *Scoparia dulcis* and *Tetracarpidium conophorum* aqueous extracts mixture at indicated doses.

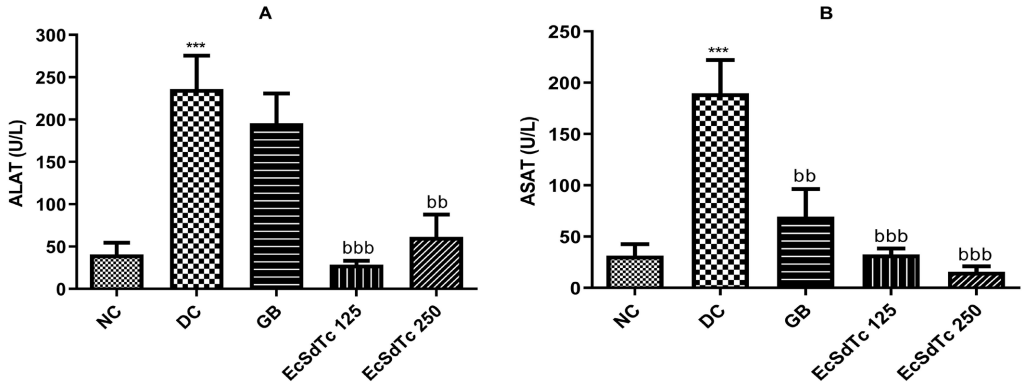

Figure 5. Serum ALAT (A) and ASAT (B) activities in plants mixture (EcSdTc)-treated diabetic rats. Each bar represents the mean ± ESM; n = 5; *** p < 0.001 vs NC; ^{bb}p < 0.01 and ^{bbb}p < 0.001 vs DC; NC = normal control; DC = diabetic control; GB = glibenclamide; EcSdTc = *Emilia coccinea*, *Scoparia dulcis* and *Tetracarpidium conophorum* aqueous extracts mixture at indicated doses.

with conventional drugs in the hope of obtaining a synergistic effect [25] [26]. Traditional healers rely more on herbal concoctions or mixtures rather than individual herbs to treat many diseases [8] [27]. However, very few studies to date have been carried out on plants mixtures for diabetes treatment. The present study evaluated the antidiabetic effects of a mixture of aqueous extracts from *Emilia coccinea* (Ec), *Scoparia dulcis* (Sd) and *Tetracarpidium conophorum* (Tc) in STZ-induced diabetic rat model. First of all, effects of the different individual extracts and their mixtures were assessed in normal rats receiving single doses, by performing oral hypoglycemic (OHGT or HGT) and glucose tolerance (OGTT or GTT) tests.

OHGT detects hypoglycemic agents, gives the first information on the action mechanism of an anti-diabetic substance (as the capacity to stimulate insulin secretion), and allows to monitor compliant drugs doses to be intake to avoid harmful hypoglycemia. GTT assesses the ability of a substance to reduce the postprandial blood glucose increase, insulin action and beta cells function [25]

[28]. Mean blood glucose levels over five- and two-hour periods were determined respectively for OHGT and GTT in normal rats by calculating the areas under respective curves (AUC). Single administration of the plant extracts (individual and mixtures) in normal rats significantly reduced the blood glucose (AUC), at doses and in the order of decreasing efficiency indicated, during the OHGT (extracts mixtures EcTc250, EcSdTc125, SdTc250, SdTc125, EcSd250 and EcSdTc250), and the GTT (individual extracts and mixtures (EcTc250, Tc125, Tc250, Ec250, EcSdTc250 and Sd250), resulting in 10 efficient treatments as EcTc250, EcSdTc125, SdTc250, SdTc125, EcSd250, EcSdTc250, Tc125, Tc250, Ec250 and Sd250.

In sum, seven (7) of the above 10 efficient treatments registered contained the *T. conophorum* (Tc) extract against 6 containing the *S. dulcis* (Sd) extract and 5 the *E. coccinea* (Ec) extract, suggesting that Tc would be probably more hypoglycemic than Ec and Sd. The drop in blood glucose observed during GTT at individual extract doses and in the order of efficacy such as Tc125 > Tc250 > Ec250 > Sd250, once again supports the high effectiveness of Tc compared to Ec and Sd, and also suggests that the aqueous extracts of Ec, Sd and Tc can reduce postprandial hyperglycaemia.

Data on effects of single administration of Sd extracts on blood glucose of normoglycemic rats are not documented. But its prolonged administration, at least for 7 days, are reported lowering the blood glucose of normal and/or diabetic rats/Human [11] [12] [13] [15] [16] [29]-[34]. Furthermore, aqueous extracts of *E. coccinea* (215 mg/kg) [19] and *T. conophorum* seed (500 mg/kg) [25] administered acutely 30 minutes prior to oral glucose load (3 mg/kg) in normal rats are reported reducing the post prandial hyperglycaemia. Moreover, the reported inversely dose-dependent efficacy of *T. conophorum* seed aqueous extract (500 mg/kg > 1000 mg/kg) [25] was also observed in the present results with the trunk bark aqueous extract (Tc 125 mg/kg > Tc 250 mg/kg in GTT), suggesting that, in view of the doses used in these two studies, the Tc bark would be to a lesser extent more effective than the fruit.

Since a whole plant extract is basically a collection of natural phytochemicals that might exert synergistic, antagonistic, additive or insignificant effects depending on the interaction between them, a potion derived from several plants extracts combination can also produce these effects with a more or less high effectiveness [8]. Interestingly, 6 of the above 10 efficient treatments were plant extracts mixtures, with EcTc250 being the most effective treatment in both acute tests (HGT and GTT), and even than the empirically used EcSdTc mixture (at both doses of 125 and 250 mg/kg), which would also suggest the synergistic or additive effects of phytochemicals from individual extracts Ec, Sd and Tc. The order of efficacy of the individual Ec, Sd and Tc extracts observed during the GTT at the dose of 250 mg/kg (Tc250 > Ec250 > Sd250) and the calculated yields of plants extraction as Ec > Tc > Sd (11.57% > 7.40% > 6.17%), could justify that greater efficacy of the EcTc250 mixture treatment in both acute tests compared

to its components (Ec and Tc) and other mixtures administrated at the same dose, and therefore support the additive or synergistic effect of the Ec and Tc components. Many studies have shown that combination of plants extracts increased the hypoglycemic potential of the individual plant extracts in normal and diabetic rats [17] [35]-[40].

Elsewhere, although EcSdTc mixture at 250 mg/kg dose has been effective in both acute tests, its low effect compared to EcTc mixture (250 mg/kg) (by 9.5% in OHGT and 8.32% in GTT) could be explained by the addition of Sd in EcTc mixture which reduced by 1/3th the quantity of 250 mg of each component Ec, Sd and Tc (*i.e.*, 83.33 mg each in the 250 mg/kg dose of EcSdTc), or of which constituents would have inhibit or antagonized the effects of Ec and/or Tc metabolites. Presence of flavonoids, tannins, saponins, steroids, phenols, glucosides and alkaloids has been reported in the Ec, Sd and parts of Tc (leaves, fruits and roots) aqueous extracts [16] [41]-[46]. Quantitative phytochemical studies showed the much higher alkaloid content in Sd aqueous extract (93 mg/g) than in Ec (0.0094 mg/g) and Tc (0.41 mg/g) aqueous extracts [47] [48] [49], which can justify the reduced effect of the EcTc mixture after addition of Sd. Alkaloids at low doses have many beneficial pharmacological effects such as in berberine's hypoglycemic effects [50], but at high doses induce cytotoxic effects in tissues (kidneys, liver, brain, etc.) via the DNA alkylation that could thus hinder proteins synthesis and possibly of insulin by the pancreatic β cells, thereby reducing its secretion. In addition, the low content of tannins in these plant extracts (Ec, Sd and Tc) would probably not have been able to precipitate the excess of alkaloids in order to neutralize their toxicity as described [50].

Nevertheless, the above effective treatments revealed in OHGT and GTT AUC values comparable to or not significantly higher than those of glibenclamide. This suggest that these treatments contain hypoglycemic bioactive metabolites which would have reduced the blood glucose probably by stimulating beta cells insulin secretion and reducing alpha cells glucagon secretion directly like glibenclamide or indirectly via incretins secretion [51]. The plant metabolites would have also stimulate blood glucose uptake and use in adipocytes and skeletal muscles [51] [52], act as an insulin analogue, inhibit intestinal glucose transport via SGlut-2 and digestive enzymes inhibition (α-amylase, β-glucosidase etc.), and/or suppress hepatic glucose production via the inhibition of gluconeogenesis enzymes synthesis [51] [53] [54]. Thus, these probable effects of aqueous extracts of Ec, Sd, Tc and even their mixtures would be important in the management of diabetes mellitus.

The experimental streptozotocin (STZ)-induced diabetic animal model is still widely used today to assess the anti-diabetic properties of natural products derived from medicinal plants [55] and/or for the improvement of diabetic complications [56]. The present study showed in diabetic control rats (DC) (STZ administered at 50 mg/kg) serum metabolic alterations (persistent hyperglycemia, increased creatininemia, triglyceridemia, total cholesterolemia, LDL-cholesterolemia,

and ALAT/ASAT activities; decreased proteinemia and HDL-cholesterolemia) and associated severe weight loss as generally seen in STZ-induced diabetes [22] [57] [58] [59], characterizing the type 1 diabetes mellitus. Indeed, the dose-dependent destruction of pancreatic β cells leading to the blood glucose increase is one of the nowadays well established and known effects of STZ [18] [22] [60]. The STZ dose of 50 mg/kg is reported destroying enough beta cells leaving a residual small number [61] [62], which justifies the use of glibenclamide in the present study as reference drug as it is reported mainly stimulating insulin secretion and probably additional pancreatic beta cells proliferation [63]. The STZ's cytotoxic effects induces β cells damage leading firstly to insulinopenia and therefore to hyperglycemia. STZ also alters hepatic and kidney cells that express the Glut-2 insulin transporter. However, STZ life being really short (Half-life of 15 minutes in the serum after intravenous injection and probable elimination within 48 hours after intraperitoneal injection), and its DNA methylating effect for this reason quickly diminishing, β cells dysfunction is therefore maintained after induction of diabetes by the persistent hyperglycemia, which is the basis for the many other metabolic alterations [64].

The empirically used EcSdTc mixture reduced significantly and more than glibenclamide the blood glucose in diabetic rats 5 h after acute administration, compared to 0 h. Interestingly, the mixture even better reduced this parameter after 21 days of treatment as effective as (125 mg/kg bw) or more than (250 mg/kg bw) glibenclamide, all compared to DC rats. Mixtures of ethanolic extracts of *Acanthus montanus*, *Emilia coccinea*, *Hibiscus rosasinensis* and *Asystasia gangetica* have also been reported reducing blood glucose in alloxan-induced diabetic rats after 30 days of treatment [17]. The mechanism of action of the EcSdTc hypoglycemic property is not known, but each of its individual plant components is reported to reduce the blood glucose in diabetic condition [19] [34] [36] [38] [65] [66] [67], suggesting that the wide variety of chemical classes among the many extracted substances of each plant indicates probable involvement of variety of mechanisms in the blood glucose reduction as reported by Gushiken *et al.* (2016) [68]. Constituents of these hypoglycemic plants as Scoparic acid D, Coxoil and Glutinol from *S. dulcis* [15] [69] [70], some flavonoids, terpenes as saponins and alkaloids from all of them are known to stimulate β cells insulin secretion *in vitro* and/or *in vivo*, to inhibit hepatic gluconeogenesis and intestinal glucose absorption trough synthesis modulation of intestinal and hepatic enzymes involved in carbohydrates metabolism, to stimulate hepatic glycogenesis, to stimulate peripheral glucose uptake and utilization, and residual pancreatic β cells regeneration [11] [38] [40] [41] [43] [44] [45] [71] [72] [73].

The insulinopenia due to β cells damage after STZ administration and the consequent hyperglycemia also subsequently contribute to the serum creatinine increase as observed in DC rats compared to NC rats. Creatinine comes from the breakdown of muscle creatine and is mostly eliminated in the kidneys by glo-

merular filtration. Its present serum increase indicates the degree of muscle creatine degradation, and therefore of muscle wasting and renal function and morphological alterations. The body mass loss observed in DC rats expresses this muscle damage marked by high creatinine and accentuated by the drop in serum protein levels, itself linked to the decrease in protein synthesis by the affected liver and lack of insulin. The decrease in serum creatinine observed in EcSdTc mixture-treated diabetic rats suggests that the plants mixture improves kidney filtration function and protects against diabetic kidney complications and muscle wasting. Moreover, the associated stabilization or increase in proteinemia reflects an improvement in hepatic function on protein metabolism. Both effects of the mixture therefore justify the observed weight increase, probably through the insulin increase or by the insulin-like effect. As both protein ingestion and carbohydrate ingestion induce insulin release and thereby stimulate creatine uptake [74], it is evident that insulin itself directly enhances both the transport rate and uptake of creatine, as shown *in vivo* [75] and *in vitro* [76] on rat skeletal muscle.

Furthermore, the increased serum quantitative lipid abnormalities and ALT/AST activities accompanying the above-described perturbations (persistent hyperglycemia, hypercreatininemia, hypoproteinemia, weight loss) in DC compared to NC rats confirm the hepatic dysfunction, as the liver is one of the main insulin targets and the main organ for glucose, lipid and protein metabolism. In addition to glucose storage and protein synthesis, the liver works synergically with adipose tissue for lipid metabolism, notably for lipolysis control under insulin action. The lipid profile in type 1 diabetes should be given special attention. Under normal conditions, insulin increases the hepatic triglycerides (TG) synthesis by increasing the fatty acids uptake in adipose cells and furthermore, inhibits lipolysis, this by decreasing hormone-sensitive lipase in adipose tissue, activating vascular lipoprotein lipase, by increasing the LDL receptor, modulating the hepatic lipase (LH) action and stimulating the LCAT activity in the liver [77]. However, the insulin depletion increases lipolysis and eventually causes hyperlipidemia [77] [78] [79], which would explain the increased triglycerides, total cholesterol and LDL-cholesterol and the decreased HDL-cholesterol observed in DC rats. These lipid abnormalities common in diabetic peoples contributed to increase the atherogenic risk index of at least 6-fold the normal, indicating increased cardiovascular risk and diabetic nephropathy as reported [77] [78]. Thus, as the liver plays an important role in glycemic balance by storing glucose or releasing it as needed, the drop or lack of insulin would direct the functioning of the liver towards its hyperglycemic metabolic processes (proteolysis and lipolysis in favor of gluconeogenesis, glycogenolysis) to the detriment of storage to meet the peripheral energy requirement due to hypo-insulinemia, leading to liver over-functioning and eventually the liver cells damage reflected by the rise in transaminases [80]. The EcSdTc mixture-induced lipid profile improvement (increased HDL-cholesterol and decreased triglycerides, total cholesterol, LDL-

cholesterol and atherogenic risk) would suggest a protective effect of EcSdTc mixture against diabetic cardiovascular complications. The plants mixture would have probably inhibited the glycogenolysis and/or gluconeogenesis enzymes such as phosphoenolpyruvate carboxykinase (flavonoids, saponins, phenols) [81], glucose-6-phosphatase (flavonoids and phenols) [82], glycogen phosphorylase (saponins) [83], hormone-sensitive lipase and activated the lipoprotein lipase thus decreasing serum levels of triglycerides. Moreover, the plants mixture would have inhibited the Acyl-Coenzyme A cholesterol Acyltransferase (ACAT) activity and the intestinal absorption of cholesterol, and/or increased its biliary excretion, thus reducing serum cholesterol (sterols and saponins) [84]. Each plant constituent of the mixture is reported reducing the lipid profile in diabetic conditions [17] [19] [32] [85]. Interestingly, the significant decrease in serum ALT/AST activities caused by the EcSdTc mixture at both doses indicates reversed hepatocellular damage processes probably due to the above-described antidiabetic properties of its numerous bioactive compounds, but also to their antioxidant effects as explained for the *Tetracarpidium conophorum* aqueous extract [86].

Oxidative stress is also most often reported in diabetes mellitus. Chronic hyperglycemia and hyperlipidemia observed in diabetes mellitus are responsible for the increased production of reactive oxygen (ROS) and nitrogen (RNS) species by oxidation of glucose, lipids and proteins, causing the depletion of antioxidant enzymes and oxidative stress-induced damage of cells [87]. The decrease in the activities or levels of SOD, Catalase, NO and GSH associated with the MDA level increase in the liver of DC rats compared to NC indicates the installation of oxidative stress after the STZ administration and consequently structural and functional damage of hepatocytes. The EcSdTc mixture at both doses and glibenclamide significantly improved these parameters in the liver of treated diabetic rats. Studies have shown that aqueous extracts of Ec, Sd and Tc administrated individually improve oxidative stress parameters in diabetic rats [10] [12] [13] [30] [31] [38] [88]; Ajiloré *et al.* (2020) [66] also reported the protective effect of *T. conophorum* seeds methanolic extract against the hyperglycemia-induced liver oxidative damage. These antioxidant effects of EcSdTc mixture could be attributed to plants polyphenolic (Flavonoids, tannins and phenols), alkaloids, terpenoids compounds and vitamins as ascorbic acid, which are likely to scavenge free radicals generated by STZ and protect cell membranes against the oxidative stress deleterious effects and consequently improve the liver function as suggested [29] [44] [88].

5. Conclusion

The plant extracts mixtures were more efficient than individual extracts in acute tests OHGT/GTT. The plants mixture EcSdTc (250 mg/kg) reduced the blood glucose in normal, glucose-loaded and diabetic rats after acute administration; Additionally, and sometimes more than glibenclamide, it improved the serum

glucose level, lipid and protein profiles, and liver function after 21 days of administration in diabetic rats. The wide variety of bioactive metabolites of EcSdTc mixture from the many extracted substances of each plant indicates probable involvement of variety of biological mechanisms, and therefore synergistic or additive effects of phytochemicals from individual extracts, which would justify the empirical use of EcSdTc mixture for diabetes treatment. However, the prolonged effects of EcTc mixture should be assessed in diabetic rats since it was the most efficient in acute tests.

Acknowledgements

We wish to express our sincere thanks to the Alexander von Humboldt Foundation, for its award of the equipment grant to one of the authors. Thanks also to Professors Paul Désiré Djomeni Dzeufiet, Theophile Dimo and Pierre Kamtchouing for their donation of reagents.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] International Diabetes Federation (IDF) (2021) IDF Diabetes Atlas. 10th Edition, International Diabetes Federation, Brussels.

[2] Abir Nachar. (2014) Evaluation of the Anti-Diabetic Effect of Medicinal Plants from the Boreal Forest and Identification of the Active Ingredients of Two Promising Species. Master's Thesis, University of Montreal, Montreal.

[3] Hassan, I.A., Abdulraheem, I., Emun, H.O. and Lawal, D.M. (2019) Synergistic Effect of Aqueous Extracts of *Croton zabensicus* and *Vernonia amygdalina* Leaves as an Antihyperglycemic Agent in an Alloxan Induced Diabetic Albino Rats. *Journal of Advances in Medicine and Medical Research*, **29**, 1-6. https://doi.org/10.9734/jammr/2019/v29i530092

[4] Ghouri, N., Purves, D., McConnachie, A., Wilson, J., Gill, J.M.R. and Sattar, N. (2013) Lower Cardiorespiratory Fitness Contributes to Increased Insulin Resistance and Fasting Glycaemia in Middle-Aged South Asian Compared with European Wen Living in the UK. *Diabetologia*, **56**, 2238-2249. https://doi.org/10.1007/s00125-013-2969-y

[5] International Diabetes Federation (IDF) (2019) IDF Diabetes Atlas. 9th Edition, International Diabetes Federation, Brussels.

[6] International Diabetes Federation (IDF) (2013) IDF Diabetes Atlas. 6th Edition, International Diabetes Federation, Brussels.

[7] Fah, L., Klotoé, J.R., Dougnon, V., Koudokpon, H., Fanou, V.B., Dandjesso, C. and Loko, F. (2013) An Ethnobotanical Study of Plants Used in the Treatment of Diabetes in Pregnant Women in Cotonou and Abomey-Calavi (Benin). *Journal of Animal and Plant Sciences*, **18**, 2647-2658.

[8] Nur Fazira, A.R., Norhayati, M., Tuan, M.T., Wan Nur, A.S., Norazlin, A., Balkis, A.T., Mohd, F.A. and Tayab, T. (2019) Antioxydant Activity and Its Interaction Effect on Polyherbal Formulations of *Nephrodium inophyllum*, *Polygonum minus*,

Annona squamosal L. and *Stevia rebaudiana*. *Journal of Advanced Research in Fluid Mechanics and Thermal Sciences*, **61**, 1-9.

[9] Latha, M. and Pari, L. (2003) Modulatory Effect of *Scoparia dulcis* in Oxidative Stress-Induced Lipid Peroxidation in Streptozotocin Diabetic Rats. *Journal of Medicinal Food*, **6**, 379-386. https://doi.org/10.1089/109662003772519958

[10] Latha, M. and Pari, L. (2004) Effect of an Aqueous Extract of *Scoparia dulcis* on Blood Glucose, Plasma Insulin and some Polyol Pathway Enzymes in Experimental Rat Diabetes. *Brazilian Journal of Medical and Biological Research*, **37**, 577-586. https://doi.org/10.1590/S0100-879X2004000400015

[11] Pari, L. and Latha, M. (2004) Antihyperglycemic Effect of *Scoparia dulcis*: Effect on Key Metabolic Enzymes of Carbohydrate Metabolism in Streptozotocin-Induced Diabetes. *Parmaceutical Biology*, **42**, 570-576. https://doi.org/10.1080/13880200490901799

[12] Latha, M., Pari, L., Sitasawad, S. and Bhonde, R. (2004) Insulin-Secretagogue Activity and Cytoprotective Role of the Traditional Antidiabetic Plant *Scoparia dulcis* (Sweet Broom Weed). *Life Science*, **75**, 2003-2014. https://doi.org/10.1016/j.lfs.2004.05.012

[13] Latha, M., Pari, L., Sitasawad, S. and Bhonde, R. (2004) *Scoparia dulcis*, a Traditional Antidiabetic Plant, Protects against Streptozotocin Induced Oxidative Stress and Apoptosis *in vitro* and *in vivo*. *Journal of Biochemical and Molecular Toxicology*, **18**, 261-272. https://doi.org/10.1002/jbt.20035

[14] Pari, L., Latha, M. and Rao, C.A. (2004) Effect of *Scoparia dulcis* Extract on Insulin Receptors in Streptozotocin-Induced Diabetic Rats: Studies on Insulin Binding to Erythrocytes. *Journal of Basic and Clinical Physiology and Pharmacology*, **15**, 223-240. https://doi.org/10.1515/JBCPP.2004.15.3-4.223

[15] Latha, M., Pari, L., Ramkumar, K.M., Rajaguru, P., Suresh, T., Dhanabal, T., Sitasawad, S. and Bhonde, R. (2009) Antidiabetic Effects of Scoparic Acid D Isolated from *Scoparia dulcis* in Rats with Streptozotocin-Induced Diabetes. *Natural Product Research*, **23**, 1528-1540. https://doi.org/10.1080/14786410902726126

[16] Zulfiker, A.H., Ripa, F.A., Rahman, M., Ullah, M.O., Hamid, K., Khan, M.R. and Rana, S. (2010) Antidiabetic and Antioxidant Effects of *Scoparia dulcis* in Alloxan Induced Albino Mice. *International journal of Pharmacology and Technology Research*, **2**, 2527-2534.

[17] Ojiako, A.O., Chikezie, P.C. and Zedechu, U.C. (2013) Serum Lipid Profile of Hyperlipidemic Rabbits (*Lepus townsendii*) Treated with Leaf Extracts of *Hibiscus rosasinensis*, *Emilia coccinea*, *Acanthus montanus* and *Asystasia gangetica*. *Journal of Medicinal Plants Research*, **7**, 3226-3231.

[18] Ajilore, B.S. and Adesokan, A.A. (2018) Antidiabetic Effects of *Tetracarpidium conophorum* Seed on Biomarkers of Diabetes-Induced Nephropathy in Rats. *Asian Pacific Journal of Tropical Medicine*, **8**, 593-597. https://doi.org/10.4103/2221-1691.248096

[19] Kamani, P.S.L., Waguia, J.K., Miaffo, D., Nchouwet, M.L., Kadji, C.L.D., Wego Kamgaing, M.T., Douho Djimeli, R.C., Mzoyem Ngnitedem, J., Kamanyi, A. and Wansi Ngnokam, S.L. (2022) Efficacy of *Emilia coccinea* Extract on Inhibition of α-Amylase Enzyme Activity and Insulin-Resistance in Dexamethasone Treated-Rats. *Metabolism Open*, **15**, Article ID: 100193. https://doi.org/10.1016/j.metop.2022.100193

[20] Reagan-Shaw, S., Nihal, M. and Nihal, A. (2017) Dose Translation from Animal to Human Studies Revisited. *The FASEB Journal*, **22**, 659-661.

https://doi.org/10.1096/fj.07-9574LSF

[21] Diehl, K.H., Hull, R., Morton, D., Pfister, R., Rabemampianina, Y., Smith, D., Vidal, J.M. and van de Vortenbosch, C. (2001) A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes. *Journal of Applied Toxicology*, **21**, 15-23. https://doi.org/10.1002/jat.727

[22] Tchamadeu, M.C., Dzeufiet, P.D.D., Blaes, N., Girolami, J.P., Kamtchouing, P. and Dimo, T. (2017) Antidiabétic Effects of Aqueous and Dichloromethane/Methanol Stem Bark Extracts of *Pterocarpus Soyauxii Taub* (*Papilionaceae*) on Streptozotocin-Induced Diabetic Rats. *Pharmacognosy Research*, **9**, 80-86. https://doi.org/10.4103/0974-8490.199767

[23] Ahmadi, S.A., Boroumand, M.A., Katayoun, G.M., Tajik, P. and Dibaj, S.M. (2008) The Impact of Low Serum Triglyceride on LDL-Cholesterol Estimation. *Archives of Iranian Medicine*, **11**, 318-321.

[24] Dobiàšovà, M. and Frohlich, J. (2001) The Plasma Parameter Log (TG/HDL-C) as an Athrogenic Index: Correlation with Lipoprotein Particle Size and Esterification Rate in ApoB-Lipoprotein-Depleted Plasma (FER_{HDL}). *Clinical Biochemistry*, **34**, 583-588. https://doi.org/10.1016/S0009-9120(01)00263-6

[25] Showande, S.J. and Bello, J.J. (2015) Remedies for Glucose Intolerance—Are Traditional Herbal Concoctions for Diabetes Effective? *Nigerian Journal of Pharmaceutical Research*, **11**, 110-118.

[26] Hasan, M.N., Sabrin, F., Rokeya, B., Khan, S.H., Ahmed, M.U., Matondo, A., Billah, M. and Akter, S. (2019) Glucose and Lipid Lowering Effects of *Enhydra fluctuans* Extract in Cadmium Treated Normal and Type-2 Diabetic Model Rats. *BMC Complementary and Alternative Medicine*, **19**, Article No. 278. https://doi.org/10.1186/s12906-019-2667-5

[27] Omar, S., Stephen, F., Khaled, K., Hassan, A., Eli, K. and Bashar, S. (2008) Maintaining a Physiological Blood Glucose Level with "Glucolevel" a Combination of Four Anti-Diabetique Plants Used in the Traditional Arab Herbal Medecine. *Evidence-Based Complementary and Alternative Medicine*, **5**, 421-428. https://doi.org/10.1093/ecam/nem047

[28] Tchamadeu, M.C., Dzeufiet, P.D.D., Nana, P., Kouambou Nouga, C.C., Ngueguim Tsofacf, F., Allard, J., Blaes, N., Pecher, C., Tack, I., Girolami, J.P., Kamtchouing, P. and Dimo, T. (2011) Acute and Sub-chronic Oral Toxicity Studies of an Aqueous Stem Bark Extract of *Pterocarpus soyauxii* Taub (*Papilionaceae*) in Rodents. *Journal of Ethnopharmacology*, **133**, 329-335. https://doi.org/10.1016/j.jep.2010.09.035

[29] Pari, L. and Latha, M. (2004) Effects of *Scoparia dulcis* (Sweet Broom Weed) Plant Extract on Plasma Anti-Oxidants in Streptozotocin-Induced Experimental Diabetes in Male Albino Wistar Rats. *Pharmazie*, **59**, 557-560.

[30] Latha, M. and Pari, L. (2005) Effect of an Aqueous Extract of *Scoparia dulcis* on Plasma and Tissue Glycoproteins in Streptozotocin-Induced Diabetic Rats. *Pharmazie*, **60**, 151-154.

[31] Pari, L. and Latha, M. (2005) Antidiabetic Effect of *Scoparia dulcis*: Effect on Lipid Peroxidation in Streptozotocin Diabetes. *General Physiology and Biopysics*, **24**, 13-26.

[32] Pari, L. and Latha, M. (2006) Antihyperlipidemic Effect of *Scoparia dulcis* (Sweet Broom Weed) in Streptozotocin Diabetic Rats. *Journal of Medicinal Food*, **9**, 102-107. https://doi.org/10.1089/jmf.2006.9.102

[33] Senadheera, S.P.A.S., Ekanayake, S. and Wanigatunge, C. (2014) Anti-Diabetic Properties of Rice-Based Herbal Porridges in Diabetic Wistar Rats. *Phytotherapy*

Research, **28**, 1567-1572. https://doi.org/10.1002/ptr.5169

[34] Senadheera, S.P.A.S., Ekanayake, S. and Wanigatunge, C. (2015) Anti-Hyperglycaemic Effects of Herbal Porridge Made of *Scoparia dulcis* Leaf Extract in Diabetics—A Randomized Crossover Clinical Trial. *BMC Complementary and Alternative Medicine*, **15**, Article No. 410. https://doi.org/10.1186/s12906-015-0935-6

[35] Sunarwidhi, A.L., Sudarsono, S. and Nugroho, A.E. (2014) Hypoglycemic Effect of Combination of *Azadirachta indica* A. Juss and *Gynura procumbens* (Lour.) Merr. Ethanolic Extracts Standardized by Rutin and Quercetin in Alloxan-Induced Hyperglycemic Rats. *Advanced Pharmaceutical Bulletin*, **4**, 613-618.

[36] Ojiako, O.A., Chikezie, P.C. and Ogbuji, A.C. (2015) Comparative Hypoglycemic Activities of Aqueous and Ethanolic Extracts of Four Medicinal Plants (*Acanthus montanus*, *Asystasia gangetica*, *Emilia coccinea and Hibiscus rosasinensis*) in Type 1 Diabetic Rats. *Journal of Intercultural Ethnopharmacology*, **4**, 228-233. https://doi.org/10.5455/jice.20150508045222

[37] Ojiako, O.A., Chikezie, P.C. and Ogbuji, A.C. (2015) Antioxidant Status of Liver and Kidney Homogenates from Hyperglycemic Rats Administered with Single and Combinatorial Herbal Formulations. *Free Radicals and Antioxidants*, **5**, 13-20. https://doi.org/10.5530/fra.2015.1.3

[38] Ojiako, O.A., Chikezie, P.C. and Ogbuji, A.C. (2016) Blood Glucose Level and Lipid Profile of Alloxan-Induced Hyperglycemic Rats Treated with Single and Combinatorial Herbal Formulations. *Journal of Traditional and Complementary Medicine*, **6**, 184-192. https://doi.org/10.1016/j.jtcme.2014.12.005

[39] Okoye, N.F., Monanu, M.O. and Ohanehi, V.O. (2017) Combined Effects of Aqueous Extracts of *Tetracarpidium Conophorum* (Walnuts) and *Vernonia Amygdalina* (*Bitter leaves*) on the Pancreas and Kidney of Alloxan Induced Diabetic Wistar Rats. *Journal of Applied Science and Environmental Management*, **21**, 893-901. https://doi.org/10.4314/jasem.v21i5.15

[40] Arise, A.K., Malomo, S.A., Acho, M.A., Ajao-Azeez, N.D. and Arise, R.O. (2023) *In vivo* Anti-diabetic Activity, Physicochemical and Sensory Properties of Kunu Enriched with African Walnut. *Food Chemistry Advances*, **2**, Article ID: 100315. https://doi.org/10.1016/j.focha.2023.100315

[41] Hayashi, T., Okamura, K., Tamada, Y., Lida, A., Fujita, T. and Morita, N. (1993) A New Chemotype of *Scoparia dulcis*. *Phytochemistry*, **32**, 349-352. https://doi.org/10.1016/S0031-9422(00)94992-6

[42] Latha, M., Ramkumar, K.M., Pari, L., Damodaran, P.N., Rajeshkannan, V. and Suresh, T. (2006) Phytochemical and Antimicrobial Study of an Antidiabetic Plant: *Scoparia dulcis* L. *Journal of Medicinal Food*, **9**, 391-394. https://doi.org/10.1089/jmf.2006.9.391

[43] Oyekale, O., Odutayo, O., Esan, E., Ogunwemimo, K., Denton, K. and Bolaji, D. (2015) Comparative Studies on Phytochemical and Proximate Composition of Four Morphologically Distinct Segments of the Conophor Seedling (*Tetracarpidium conophorum* Hutch. & Dalziel). *Brazilian Journal of Biological Sciences*, **2**, 91-100.

[44] Nwachukwu, V.A., Udedi, S.C., Ezeonu, F.C., Bartholomew, I.C.B., Ezeanyanaso, C.S. and Elemo, G.N. (2017) Bioactive Agents, Nutriceuticals Potentials, Phytochemistry and Food Value of *Emilia coccinea* Leaf. *Journal of Complementary and Alternative Medical Research*, **4**, 1-15. https://doi.org/10.9734/JOCAMR/2017/29435

[45] Mac Donald, I., Charles, O.A. and Benjamin, O.G. (2021) Phytochemistry, *in-vitro* Antioxidant, Microbicidal, Anti-Ulcerogenic and Biosafety Potential of *Emilia coccinea* Aqueous Extract in Animal Models. *Algerian Journal of Bioscience*, **2**, 67-77.

[46] Aneke, F., Offor, C., Ogbonna, B.O., Ejim, C.E., Nwankwo, O.L., Ele, G.N. and Ikebudu, C.C. (2016) Hypoglycemic Effect of the Methanolic Leaf Extract of *Tetracapidium conophorus* in Alloxane-Induced Diabetic Rat. *Asian Journal of Medical and Health Research*, **1**, 45-57. https://www.researchgate.net/publication/303541891

[47] Ayoola, P.B., Adeyeye, A., Onawumi, O.O. and Faboya, O.O.P. (2011) Phytochemical and Nutriment Evaluation of *Tetracarpidium conophorum* (Nigerian Walnut) Root. *International Journal of Research and Reviews in Applied Sciences*, **7**, 197-208.

[48] Unegbu, C.C., Obina, A., Amaralam, E.C. and Anyanwu, O.O. (2017) Evaluation of Phytochemical Contents of *Emilia coccinea* Leaves. *Journal of Medicinal Botany*, **1**, 47-50. https://doi.org/10.25081/jmb.2017.v1.817

[49] Bulama, I., Kabara, H.T., Kyari, S.A., Kano, A.M., Awwal, S.M., Ngulde, S.I. and Omeh, I.J. (2019) Qualitative and Quantitative Phytochemical Analysis of the Leaves of *Scoparia dulcis*. *Vom Journal of Veterinary Sciences*, **14**, 108-113.

[50] Christophe, B. (2016) Consoude: Toxicité des alcaloïdes. https://www.altheaprovence.com

[51] Shehadeh, M.B., Suaifan, G.A.R.Y. and Abu-Odeh, A.M. (2021) Plants Secondary Metabolites as Blood-Glucose Lowering Molecules. *Molecules*, **26**, Article 4333. https://doi.org/10.3390/molecules26144333

[52] Akhtar, N., Khan, B.A., Majid, A., Khan, H.M., Gulfishan, M.T. and Saeed, T. (2011) Pharmaceutical and Biopharmaceutical Evaluation of Extracts from Different Plant Parts of Indigenous Origin for their Hypoglycemic Responses in Rabbits. *Acta Poloniae Pharmaceutica*, **68**, 919-925.

[53] Nerurkar, P.V., Lee, Y.K. and Nerurkar, V.R. (2010) *Momordica charantia* (Bitter Melon) Inhibits Primary Human Adipocyte Differentiation by Modulating Adipogenic Genes. *BMC Complementary and Alternative Medicine*, **10**, Article No. 34. https://doi.org/10.1186/1472-6882-10-34

[54] Singh, J., Cumming, E., Manoharan, G., Kalasz, H. and Adeghate, E. (2011) Medicinal Chemistry of the Anti-Diabetic Effects of *Momordica charantia*: Active Constituents and Modes of Actions. *The Open Medicinal Chemistry Journal*, **5**, 70-77. https://doi.org/10.2174/1874104501105010070

[55] Fröde, T.S. and Medeiros, Y.S. (2008) Animal Models to Test Drugs with Potential Antidiabetic Activity. *Journal of Ethnopharmacology*, **115**, 173-183. https://doi.org/10.1016/j.jep.2007.10.038

[56] Tesch, G.H. and Allen, T.J. (2007) Methods in Renal Research: Rodent Models of Streptozotocin-Induced Diabetic Nephropathy. *Journal of Nephrology*, **12**, 261-266. https://doi.org/10.1111/j.1440-1797.2007.00796.x

[57] Roglic, G. (2016) WHO Global Report on Diabetes: A Summary. *International Journal of Non-Communicable Diseases*, **1**, 3-8. https://doi.org/10.4103/2468-8827.184853

[58] Evans, R., Lithander, F., Frese, M., Cunnigham, J. and Mills, K. (2015) Fructose Substitution of Glucose or Sucrose in Food for Normoglycaemic Persons or People with Impaired Glucose Tolerance or Diabetes. *Cochrane Database of Systematic Review*, **8**, CD011840. https://doi.org/10.1002/14651858.CD011840

[59] Balamurugan, K., Nishanthini, A. and Molan, R. (2014) Antidiabetic and Antihyperlipidaemic Activity of Ethanol Extract of *Melastoma malabathricum* Linn. Leaf in Alloxan Induced Diabetic Rats. *Asian Pacific Journal of Tropical Biomedicine*, **4**, S442-S448. https://doi.org/10.12980/APJTB.4.2014C122

[60] Noor, A., Gunasekaran, S. and Vijayalakshmi, M.A. (2017) Improvement of Insulin

Secretion and Pancreatic β-Cell Function in Streptozotocin-Induced Diabetic Rats Treated with *Aloe vera* Extract. *Pharmacognosy Research*, **9**, S99-S104. https://doi.org/10.4103/pr.pr_75_17

[61] Ar'Raiab, A. and Ahren, B. (1993) Long-Term Diabetogenic Effect of Streptozotocin in Rats. *Pancreas*, **8**, 50-57. https://doi.org/10.1097/00006676-199301000-00011

[62] Mostafavinia, A., Amini, A., Ghorishi, S.K., Pouriran, R. and Bayat, M. (2016) The Effects of Dosage and the Routes of Administrations of Streptozotocin and Alloxan on Induction Rate of Type 1 Diabetes Mellitus and Mortality Rate in Rats. *Laboratory Animal Research*, **32**, 160-165. https://doi.org/10.5625/lar.2016.32.3.160

[63] Guiot, Y., Henquin, J.C. and Rahier, J. (1994) Effects of Glibenclamide on Pancreatic β-Cell Proliferation *in vivo*. *European Journal of Pharmacology*, **261**, 157-161. https://doi.org/10.1016/0014-2999(94)90314-X

[64] Wu, J. and Yan, L.J. (2015) Streptozotocin-Induced Type 1 Diabetes in Rodents as a Model for Studying Mitochondrial Mechanisms of Diabetic β Cell Glucotoxicity. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, **8**, 181-188. https://doi.org/10.2147/DMSO.S82272

[65] Ajilore, B.S., Olorunnisola, O.S. and Owoade, A.O. (2020) *Tetracarpidium conophorum* (African Walnut) Seeds Protects against Diabetes-Induced Liver Damage in Rats Treated with Streptozotocin. *Romanian Journal of Diabetes, Nutrition and Metabolic Diseases*, **27**, 135-145.

[66] Ajilore, B.S., Olorunnisola, O.S. and Owoade, A.O. (2021) *Tetracarpidium conophorum* Seed Extract Reduces Intestinal Absorption, and Increases Cellular Trapping of Glucose. *Bulletin of the National Research Centre*, **45**, Article No. 115. https://doi.org/10.1186/s42269-021-00574-2

[67] Ajilore, B.S., Olorunnisola, O.S. and Owoade, A.O. (2021) *Tetracarpidium conophorum* Seed Extract Improves Markers of Diabetic Disease Progression in Streptozotocin-Induced Diabetic Rats. *Phytomedicine Plus*, **1**, Article ID: 100091. https://doi.org/10.1016/j.phyplu.2021.100091

[68] Gushiken, L.F., Besera, F.P., Rozza, A.L., Bérgamo, P.L., Bérgamo, D.A. and Pellizzon, C.H. (2016) Chemical and Biological Aspects of Extracts from Medicinal Plants with Antidiabetic Effects. *The Review of Diabetes Studies*, **13**, 96-112. https://doi.org/10.1900/RDS.2016.13.96

[69] Sharma, K.R., Adhikari, A., Hafizur, R.M., Hameed, A., Raza, S.A., Kalauni, S.K., Miyazaki, J.I. and Choudhary, M.I. (2015) Potent Insulin Secretagogue from *Scoparia dulcis* Linn of Nepalese Origin. *Phytotherapy Research*, **29**, 1672-1675. https://doi.org/10.1002/ptr.5412

[70] Ali, A., Haq, F.U., Arfeen, Q.U., Sharma, K.R., Adhikari, A. and Musharraf, S.G. (2017) Sensitive Quantification of Coixol, a Potent Insulin Secretagogue, in *Scoparia dulcis* Extract Using High Performance Liquid Chromatography Combined with Tandem Mass Spectrometry and UV Detection. *Biomedical Chromatography*, **31**, e3964. https://doi.org/10.1002/bmc.3964

[71] Okhale, S.E, Amanabo, M.O., Jegede, I.A., Egharevba, H.O., Muazzam, I.W. and Kunle, O.F. (2010) Phytochemical and Pharmacognostic Investigation of Antidiabetic *Scoparia dulcis* Linn (*Scrophulariaceae*) Whole Plant Grown in Nigeria. *Researcher*, **2**, 7-16.

[72] Christi, V.E.I. and Fogarty, B.N.I. (2021) Phytochemical Study on the Extract of *Scoparia dulcis* LINN. Leaves. *International Journal of Pharmaceutical Sciences and Research*, **12**, 4371-4378.

[73] Jiang, Z., Sung, J., Wang, X., Zhang, Y., Wang, Y., Zhou, H. and Wen, L. (2021) A

Review on the Phytochemistry and Pharmacology of the Herb *Scoparia dulcis* L. for the Potential Treatment of Metabolic Syndrome. *Royal Society of Chemistry Advances*, **11**, 31235-31259. https://doi.org/10.1039/D1RA05090G

[74] Steenge, G.R., Simpson, E.J. and Greenhaff, P.L. (2000) Protein- and Carbohydrate-Induced of Augmentation of Whole Body Creatine Retention in Humans? *Journal of Applied Physiolology*, **89**, 1165-1171. https://doi.org/10.1152/jappl.2000.89.3.1165

[75] Koszalka, T.R., Andrew, C.L. and Brent, R.L. (1972) Effect of Insulin on the Uptake of Creatine-1-^{14}C by Skeletal Muscle in Normal and X-Irradiated Rats. *Proceedings of the Society for Experimental Biology and Medicine*, **139**, 1265-1271. https://doi.org/10.3181/00379727-139-36344

[76] Haugland, R.B. and Chang, D.T. (1975) Insulin Effect on Creatine Transport in Skeletal Muscle. *Proceedings of the Society for Experimental Biology and Medicine*, **148**, 1-4. https://doi.org/10.3181/00379727-148-38464

[77] Vergès, B. (2013) Lipides et Diabète de Type 1 [Lipids and Type 1 Diabetes]. *Médecine des Maladies Métaboliques*, **7**, 437-442. https://doi.org/10.1016/S1957-2557(13)70533-9

[78] Chen, S.C. and Tseng, C.H. (2013) Dyslipidemia, Kidney Disease, and Cardiovascular Disease in Diabetic Patients. *The Review of Diabetic Studies*, **10**, 88-100.

[79] Roh, S.G., Kim, J.H. and Choi, W.C. (2009) Antidiabetic Synergetic Effects of Plant Extract-Mixtures in Streptozotocin-Diabetes Rats. *Journal of Life Science*, **19**, 334-342. https://doi.org/10.5352/JLS.2009.19.3.334

[80] Ferré, P. (2005) Nouvelle: Action et Sécrétion de L'insuline, Double Jeu pour les Canaux Potassiques. *Médecine Science*, **21**, 694-696. https://doi.org/10.1051/medsci/2005218-9694

[81] Ji, X.Y., Shi, S., Liu, B., Shan, M.X., Tang, D.L., Zhang, W.T., Zhang, Y., Zhang, L.L., Zhang, H.M., Lu, C. and Wang, Y.Y. (2019) Bioactive Compounds from Herbal Medicines to Manage Dyslipidemia. *Biomedicine and Pharmacotherapy*, **118**, Article ID: 109338. https://doi.org/10.1016/j.biopha.2019.109338

[82] Oakenfull, D. and Sidhu, G.S. (1990) Could Saponins be a Useful Treatment for Hypocholesterolemic? *European Journal of Clinical Nutrition*, **44**, 79-88.

[83] Sarkhail, P., Rahmanipour, S., Fadyevatan, S., Mohammadirad, A., Dehghan, G., Amin, G., Shafiee, A. and Abdollahi, M. (2007) Antidiabetic Effect of *Phlomis anisodonta*: Effects on Hepatic Cells Lipid Peroxidation and Antioxidant Enzymes in Experimental Diabetes. *Pharmacological Research*, **56**, 261-266. https://doi.org/10.1016/j.phrs.2007.07.003

[84] Analike, R.A., Ahaneku, J.E., Njoku, M.C., Ahaneku, G.I., Ezeugwunne, I.P. and Ogbodo, E.C. (2017) Effets of *Tetracarpidium conophorum* Nigeria Walnuts on Blood Lipids, Lipoproteins and Glucose Values in Adult Nigerians. *International Journal of Innovative Research and Advanced Studies*, **4**, 67-71.

[85] Lepzem, N.G. and Togun, R.A. (2017) Antidiabetic and Antioxidant Effects of Methanolic Extracts of Leaf and Seed of *Tetracarpidium conophorum* on Alloxan-Induced Diabetic Wistar Rats. *Journal of Biomedical Science and Engineering*, **10**, 402-420. https://doi.org/10.4236/jbise.2017.108031

[86] Kanu, A.M., Kalu, J.E. and Okorie, C. (2015) Nutritional and Health Values of African Walnut (*Tetracarpidium conophorum*). *International Journal of Scientific and Technology Research*, **4**, 215-220.

[87] Pitocco, D., Tesauro, M., Alessandro, R., Ghirlanda, G. and Cardillo, C. (2013) Oxidative Stress in Diabetes: Implication for Vascular and Other Complications.

International Journal of Molecular Sciences, **14**, 21525-21550.
https://doi.org/10.3390/ijms141121525

[88] Oriakhi, K., Uadia, P.O. and Eze, I.G. (2018) Hepatoprotective Potentials of Methanol Extract of *Tetracarpidium conophorum* Seeds of Carbon Tetrachoride Induced Liver Damage in Wistar Rats. *Clinical Phytoscience*, **4**, Article No. 25.
https://doi.org/10.1186/s40816-018-0085-8