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Research Article

Antidiabetic and Antioxidant Effect of Ethanolic Extract of Propolis from Meiganga (Cameroon) on Type 2 Diabetes in Rats

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Abstract

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia. In Cameroon, the traditional treatment of this pathology is based on the use of Propolis. The present study aims to evaluate the antidiabetic and antioxidant properties of the ethanoic extract of Meiganga propolis (Adamawa Region, Cameroon). To confirm the different properties of this extract, the glycaemia, lipid profile and oxidative stress parameters of different groups of animals were assessed in a type 2 diabetes model induced by the Hypercaloric Sucrose Diet combined with dexamethasone. Simultaneous administration of the ethanolic extract of Meiganga propolis (EEMP 300 mg/kg) and the hypercaloric sucrose diet to rats for 30 days prevented a significant increase in fasting blood glucose levels compared with animals in the diabetic control group, whose fasting blood glucose levels were 78.25 ± 2.29 and 156.5 ± 2.75 mg/dL respectively on day 30. In terms of lipid profile, the administration of propolis extract (300 mg/kg) prevented a significant increase in LDL-cholesterol and triglyceride levels compared with animals in the diabetic control groups. The values were 27 ±2.71 mg/dL and 97.8 ±2.92 mg/dL for LDL-cholesterol. In terms of oxidative stress, simultaneous administration of propolis extract (300 mg/kg) and a high calorie diet to rats for 30 days prevented a significant increase in malondialdehyde (MDA) and increased superoxide dismutase (SOD) levels compared with animals in the diabetic control group. Values for this superoxide dismutase in the liver were 81.72 U/g of organ for EEMP 300 mg/kg and 58.6 U/g of organ in the liver of diabetic rats. These results justify the use of ethanoic extract of Meiganga propolis in the prevention of type 2 diabetes in Cameroon.

Keywords

Type 2 Diabetes, Oxidative Stress, Propolis, MACAPOS, Dexamethasone

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1. Introduction

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia resulting from a defect in insulin secretion and/or insulin action [1]. Diabetes is affecting more and more people around the world, and is now a major public health problem. According to the International Diabetes Federation (IDF), there will be 537 million diabetics aged between 20 and 79 in 2021. This number is expected to rise to 643 million by 2030 and 783 million by 2045. In Africa, the number is 24 million and is expected to reach 55 million by 2045 [2]. In Cameroon, the prevalence of this disease reaches 6% of individuals aged between 20 and 79 [3]. There are two main types of diabetes mellitus: type 1 diabetes mellitus (T1DM), resulting from an autoimmune reaction against the β-cells of the pancreas, and type 2 diabetes mellitus (T2DM), characterized by insulin resistance [4]. T2DM is the most common form of diabetes, accounting for around 90% of cases worldwide [2]. This alarming figure is due to changes in lifestyle [5]. Failure to manage diabetes leads to complications linked to a number of factors, including chronic hyperglycemia, enzymatic glycation of proteins and oxidative stress [6]. Oxidative stress is at the root of diabetic complications such as retinopathy, neuropathy, cardiovascular disease and ulcers [7]. Current therapies for managing diabetes mellitus help to maintain blood glucose levels around normal and prevent the onset of complications. In the case of T2DM, hygienic and dietary measures are the first components of treatment, with the introduction of a low-calorie diet rich in dietary fiber; unsaturated fats, and regular physical activity [8]. If dietary hygiene measures are ineffective, oral antidiabetics (Metformin, glibenclamide, etc.) may be considered. However, the use of these drugs has worrying side effects, such as nausea, vomiting, diarrhea, anemia, weight gain, increased risk of heart failure and fractures, fever, hypoglycemia and reduced appetite [9-13]. Managing diabetes without side effects remains a research challenge [14]. Natural products are considered an effective alternative for diabetes management, due to their efficacy and safety [15]. Apitherapy is a practice used in traditional medicine that encourages the use of bee products for therapeutic purposes [16]. These products include propolis, a natural resinous and highly adhesive substance made by bees (*Apis mellifera L.*) from the buds and leaves of certain plants, mixed with pollen and enzymes secreted by the bees [17]. Bees use propolis as an antiseptic or as a glue to seal the spaces between bees nests, to embalm dead intruders and to protect the hive from contamination [18]. The chemical composition of propolis varies according to the geographical area in which it is harvested and the type of flora in that region. Today, more than 300 compounds, including flavonoids, terpenoids, steroids, sugars, vitamins and amino acids have been detected in propolis [19]. Propolis is used in traditional medicine in many parts of the world for a variety of effects, including antibacterial, antiviral, anti-inflammatory, anticancer and anti-diabetic [20, 21, 22, 23, 24]. The aim of our study was therefore to evaluate the effects of Ethanolic Extract of Meiganga Propolis (EEMP) from the Adamawa region of Cameroon on T2DM induced by a hypercaloric sucrose diet (HDS) combined with an injection of dexamethasone in rats.

2. Materials and Methods

2.1. Animal Material

The experiments were carried out on Wistar rats (Rattus norvegicus) weighing between 160 and 190 g from the animal house of the Faculty of Science at the University of Ngaoundere (Cameroon). The animals were reared under ambient temperature conditions, subjected to a 12h/12h light/dark cycle, with free access to water and food of standard composition. The experimental protocol was that approved by the Institutional Animal Ethics Committee (reference no. FWIRB 00001954).

2.2. Source of Propolis

The propolis used in this study came from Meiganga, Adamawa Region, Cameroon. It was harvested in June 2020 and made available to us by Professor Tchuenguem-Fohouo Fernand Nestor of the University of Ngaoundere, a member of the BFM Association (Bee-Flower-Man) located in Ngaoundere.

2.3. Extraction

Propolis was extracted using the method described by Fatiha (2010) with a few modifications [25]. Thus, one hundred grams (100 g) of propolis were macerated in 500 mL of ethanol (70 %), and kept at room temperature for 72 h under mechanical agitation, protected from light followed by heating at 50 ℃ for 10 min. The macerate obtained was filtered on Wathman n°4 filter paper, then concentrated in a rotary evaporator (at 40 ℃) and dried in an oven at 40 ℃ for 48 hours. After drying, the Ethanolic Extract of the Propolis obtained was stored in a refrigerator at 4 ℃.

2.4. Evaluation of the Antidiabetic Properties of EEMP

2.4.1. Evaluation of the Effect of EEMP on the Oral Glucose Tolerance Test (OGTT) in Rats

For this test, 30 normal glycemic rats were fasted for 12 h and then divided into 6 groups of 5 animals each followed by

fasting blood glucose measurements. Once the fasting blood glucose was taken, group 1 was given distilled water, groups 2, 3, 4 and 5 were treated with EEMP at doses of 32.5, 75, 150 and 300 mg/kg body weight respectively and group 6 was given metformin at 20 mg/kg body weight. 30 minutes after administration of these different treatments, D-glucose (3 g/kg body weight) was administered orally to all rats. Blood glucose levels were read using a strip mounted on an AC-CU-CHEK glucometer and from a notch made in the distal end of the tail of each rat at 30, 60, 120 and 180 min after D-glucose treatment, respectively.

2.4.2. Evaluation of the Preventive Effect of EEMP in Rats Fed a Hypercaloric Sucrose Diet Combined with Dexamethasone

Induction of type 2 diabetes

The T2DM model was induced for thirty (30) days using normoglycemic male rats on a HSD following the MACAPOS (Maize Cassava palm Oil and sucrose) model described by Kamgang *et al* (2005) composed of: 70 to 75% carbohydrate, 10 to 15% lipid and 15 to 20% protein (Table 1) simultaneously with daily intraperitoneal administration of dexamethasone at a dose of 0.2 mg/kg body weight [26, 27].

Table 1. *Different diet compositions and their nutritional values [28].*

Groups	Maize	Wheat	Steeped cassava	Sucrose	Soya bean	Fish flour	Cabbage palm cake	Palm oil	Bones flour	Vitamins complex	Energy (kcal/kg)
ND	250	400	-	-	150	100	80	-	10	10	3400
HSD	290	200	100	200	100	30	-	60	10	10	4300

ND: normal (or standard) diet; HSD: hypercaloric sucrose diet

2.4.3. Animal Distribution and Treatment

Thirty (30) male rats were acclimatised, divided into six (06) groups of five (05) rats each and treated daily for one month orally as follows:

1) Group I (Normal control (NC)): ND + 10 ml/kg distilled water;
2) Group II (Diabetic Control (DC)): HSD + 0.2 mg/kg of dexamethasone + 10 ml/kg distilled water;
3) Group III: HSD + 0.2 mg/kg of dexamethasone + EEMP 75 mg/kg;
4) Group IV: HSD + 0.2 mg/kg of dexamethasone + EEMP 150 mg/kg;
5) Group V: HSD + 0.2 mg/kg of dexamethasone + EEMP 300 mg/kg;
6) Group VI (MET): HSD + 0.2 mg/kg of dexamethasone + 20 mg/kg Metformin.

2.4.4. Collection of Blood and Organs

After one month of treatment, the animals were sacrificed by cervical dislocation after anaesthesia by intraperitoneal injection of ketamine (50 mg/kg body weight) and diazepam (10 mg/kg body weight). Arteriovenous blood collected in dry tubes was centrifuged at 3000 rpm for 15 minutes. The collected serum was stored at -20 ℃ for subsequent assays of biochemical parameters. The liver, kidney and heart were removed, rinsed in 0.09 % physiological saline and weighed to determine relative organ weights using the formula:

$$\text{Relative organ weight} = \frac{\text{organ weight}}{\text{animal weight}} \times 100 \quad (1)$$

Tris-HCl buffer (50 mmol) was used for homogenates (20% w/v) from liver and kidney. Each homogenate was centrifuged at 3000 rpm for 15 minutes and the supernatant collected was stored at -20 ℃ for assessment of oxidative stress parameters.

2.4.5. Biochemical Analysis

The lipid profile (cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol), urea, bilirubin and proteins were determined using Fortress kits (United Kingdom), transaminase activities (ALAT, ASAT) were determined using the method of Reitman and Frankel (1957) and creatinine levels were determined using the method of Bartels *et al*. (1969) [29, 30]. Protein concentration was determined using the method described by Gornal *et al*. (1949) [31]. Malondialdehyde (MDA) was determined using the procedure of Wills (1996) [32], SOD was determined using the method of Misra and Fridovish (1972) [33], while reduced glutathione (GSH) was assessed using the method of Ellman (1959) [34]. Catalase activity (CAT) was determined using the method of Sinah *et al* (1972) [35]. The atherogenic index (AI) was calculated using the formula of Niroumand *et al* (1959) [36]:

$$\text{AI} = \text{Log}\ \frac{\text{Triglycérides}}{\text{HDL}-\text{c}} \quad (2)$$

2.4.6. Statistical Analysis

The results obtained were analysed using GraphPad Prism 8.0.1 software and expressed as mean ± standard error of the mean (SEM). An analysis of variance ANOVA One way and ANOVA Two way followed by the Turkey test

were performed to determine the difference between the batches. Values were considered statistically significant at $p<0.05$.

3. Results

3.1. Effect of EEMP on the Oral Glucose Tolerance Test in Normal Rats

Before the administration of glucose (T0), blood glucose levels showed no significant difference between the different groups of animals. 30 min after oral administration of glucose, blood glucose levels reached their highest peak observed in all groups of rats. This hyperglycaemia was maintained for up to 120 min in the groups treated with distilled water and at doses of 32.5 and 75 mg/kg body weight (Figure 1). The blood glucose values for the different peaks were: 142.6 ± 5.15; 131.2 ± 4.86; 134 ± 5.41; 147.2 ± 5.18; 144.6 ± 5.98; 86 ± 2.26 mg/dL respectively for distilled water, EEMP (32.5; 75; 150 and 300 mg/kg) and metformin (20 mg/kg). At 120 min after glucose administration, EEMP (150 and 300 mg/kg) caused a significant reduction ($p<0.001$) in blood glucose levels compared with the group treated with distilled water. These blood glucose values were: 97 ± 5.77 and 77.8 ± 6.32 mg/dL for the 150 and 300 mg/kg doses respectively. In the case of metformin (20 mg/kg), the pic blood glucose level was significantly ($p<0.001$) lower than in the group treated with distilled water. This value was 74.2 ± 2.29 mg/dL.

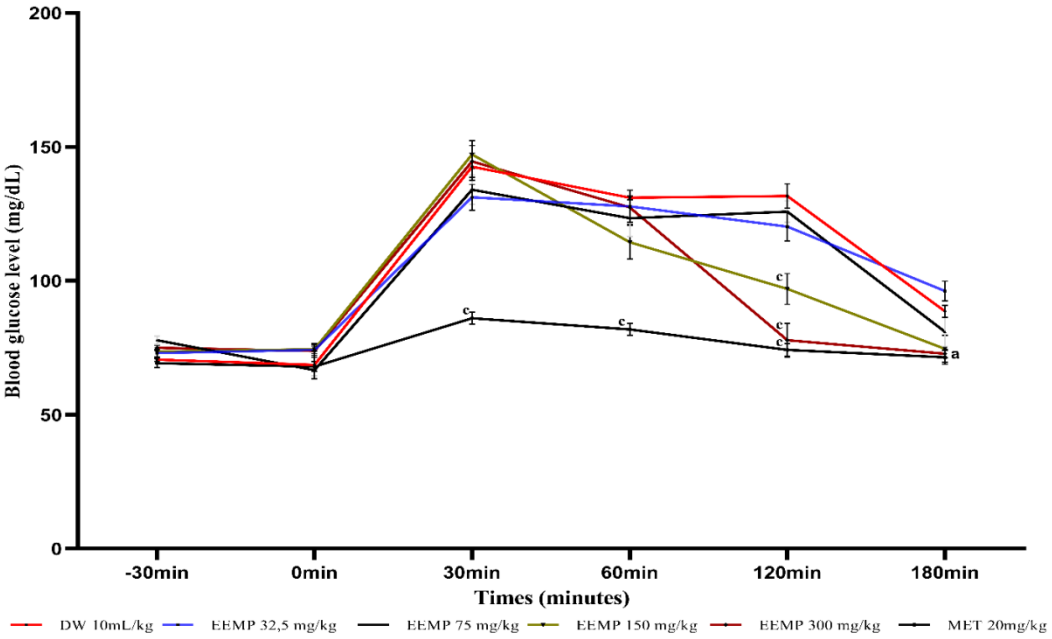


Figure 1. *Effect of EEMP on the oral glucose tolerance test in normal rats.*

Each bar represents the mean of each group ±SEM (n = 5). [a]P<0.05; [b]P<0.01; [c]P<0.001; significant difference compared with the control group; DW: distilled water; EEMP: ethanolic extract of Meiganga propolis; MET: metformin.

3.2. Effect of EEMP on Fasting Blood Glucose Levels in Rats Fed by HSD and Dexamethasone Administration

The effect of EEMP on fasting blood glucose levels in rats fed by HSD is shown in Figure 2. This figure shows that the blood glucose levels of rats in the diabetic control group treated with distilled water increased significantly ($p < 0.001$) from the start of diabetes induction to the end of induction (day 30) compared with those of rats in the normal control group. Blood glucose levels in the diabetic control group rose from 88.2 ± 5.26 mg/dL on day 0 to 156.5 ± 2.75 mg/dL on day 30. Simultaneous administration of the HSD combined with EEMP prevented a significant increase in fasting blood glucose levels. EEMP (300 mg/kg) and metformin (20 mg/kg) also prevented a rise in blood glucose levels compared with diabetic control rats. The blood glucose values were: 86.2 ± 6.48 mg/dL and 86.6 ± 5.04 on day 0; 78.25 ± 2.29 mg/dL and 80.75 ± 3.38 mg/dL on day 30 for EEPM (300 mg/kg) and metformin (20 mg/kg) respectively.

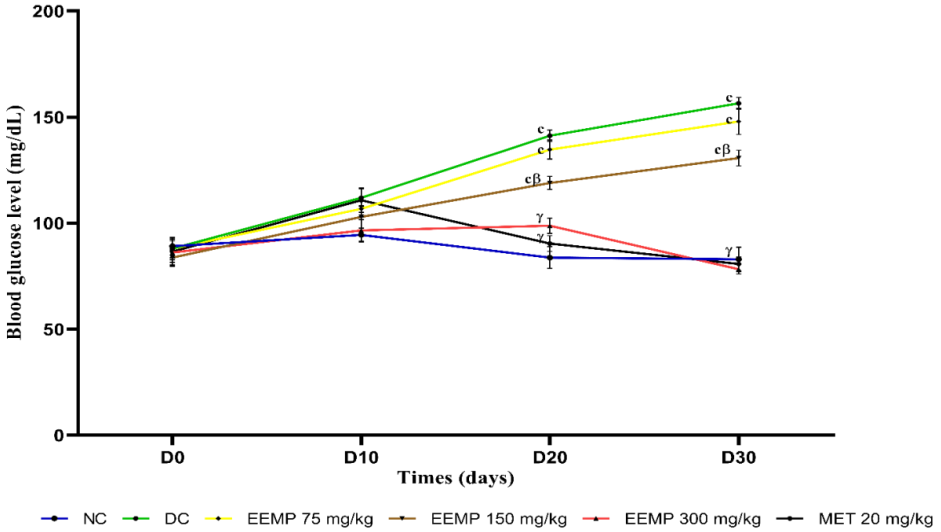


Figure 2. *Effect of EEMP on fasting blood glucose level in rats fed by hypercaloric sucrose diet.*

Each bar represents the mean of each group ± SEM (n = 5). $^{a}P<0.05$; $^{b}P<0.01$; $^{c}P<0.001$: significant difference compared with the normal control group. $^{\alpha P}<0.05$; $^{\beta P}<0.01$; $^{\gamma}P<0.001$: significant difference compared with the diabetic control group. NC: normal control; DC: diabetic control; EEMP: Ethanolic Extract of Meiganga Propolis; MET: metformin.

3.3. Effect of the EEMP on the Body Weight of Rats Fed a Hypercaloric Sucrose Diet

Figure 3 shows the effect of the simultaneous administration of the sucrose hypercaloric diet and the EEMP on the body weight of the rats. It can be seen from this figure that the body weight of the animals that received a glucocorticoid in addition to the HSD fell. The animals that received EEMP (300 mg/kg) and metformin (20 mg/kg) did not show a significant reduction compared with the animals in the normal control group. However, the reduction remained significant ($P<0.01$) compared with animals in the diabetic control group.

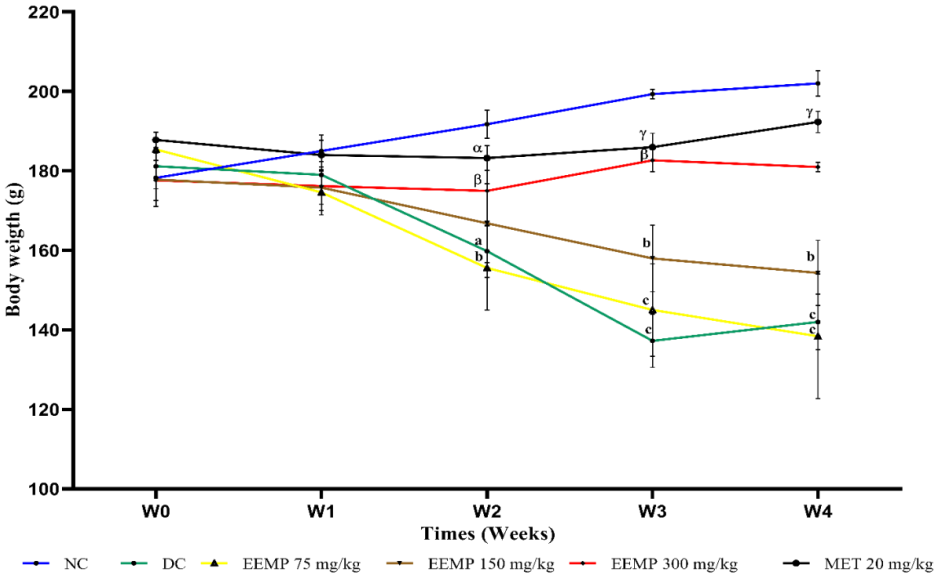


Figure 3. *Effect of EEMP on body weigth in rats fed by hypercaloric sucrose diet.*

Each bar represents the mean of each group ± SEM (n = 5). $^{a}P<0.05$; $^{bP}<0.01$; $^{cP}<0.001$: significant difference compared with the normal control group. $^{\alpha}P < 0.05$; $^{\beta}P < 0.01$; $^{\gamma}P < 0.001$: significant difference compared with the diabetic control group. NC: normal control; DC: diabetic control; EEMP: Ethanolic Extract of Meiganga Propolis; MET: metformin.

3.4. Effect of EEMP on Relative Organ Weights in Rats Fed a Hypercaloric Sucrose Diet

Table 2 shows the effect of the combined administration of HSD and the EEMP on relative organ weights in rats. The table shows that the administration of this diet combined with the extract did not cause any morphological changes in the animals' organs.

Table 2. *Effect of EEPM on relative organ weights in rats fed a hypercaloric sucrose diet.*

Groups	Relative organ weights (g/100g body weight)		
	Heart	Liver	Kidney
NC	0,30 ±0,02	4,18 ±0,56	0,60 ±0,02
DC	0,42 ±0,04	5,41 ±0,49	0,68 ±0,05
EEMP 75 mg/kg	0,30 ±0,06	4,12 ±0,02	0,60 ±0,09
EEMP 150 mg/kg	0,38 ±0,05	4,23 ±0,52	0,69 ±0,01
EEMP 300 mg/kg	0,32 ±0,02	4,29 ±0,42	0,62 ±0,26
MET 20mg/kg	0,32 ±0,08	5,09 ±0,25	0,71 ±0,04

Results are presented as mean ± SEM (n = 5). $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$: significant difference compared with the normal control group. $^{\alpha}P<0.05$, $^{\beta}P<0.01$, $^{\gamma}P<0.001$: significant difference compared with the diabetic control group. NC: normal control; DC: diabetic control; EEMP: Ethanolic Extract of Meiganga Propolis; MET: metformin.

3.5. Effect of EEMP on the Lipid Profile and Atherogenic Index of Rats

Simultaneous administration of the hypercaloric sucrose diet and distilled water resulted in a significant increase in triglyceride, total cholesterol and LDL-cholesterol levels and a significant reduction in HDL-cholesterol levels ($p<0.001$) in the diabetic control group compared with the normal control, followed by an increase in the atherogenic index (Table 3). However, daily administration of either EEMP at a dose of 300 mg/kg or metformin at 20 mg/kg for 30 days, combined with a hypercaloric sucrose diet, prevented a significant increase ($p<0.001$) in triglyceride, total cholesterol and LDL-cholesterol levels and a significant increase in HDL-cholesterol levels ($p<0.001$) compared with the diabetic control group, as well as an improvement in the atherogenic index. This index fell from 1.14 ± 0.11 in rats in the diabetic control group to 0.31 ± 0.02 and 0.31 ± 0.01 respectively in rats treated with EEMP 300 mg/kg and metformin 20 mg/kg.

Table 3. *Effects of EEMP on lipid profile and atherogenic index in rats fed a hypercaloric sucrose diet.*

Groups	TG (mg/dL)	TC (mg/dL)	HDL- c (mg/dL)	LDL-c (mg/dL)	AI
NC	87,5 ±5,12	90,4 ±4,01	44,2 ±4,36	28,7 ±7	0,35 ±0,06
DC	174 ±4,14^{c}	131 ±3,42^{c}	16,2 ±1,59^{c}	80 ±2,6^{c}	1,14 ±0,11^{a}
EEMP 75 mg/kg	165,8 ±7,05^{c}	127,2 ±4,4^{c}	25 ±2,77^{c}	68,84 ±3,08^{c}	0,82 ±0,09^{b}
EEMP 150 mg/kg	119 ±5,04$^{c\gamma}$	119 ±2,54$^{c\gamma}$	56,6 ±4,06$^{a\gamma}$	38,1 ±2,87$^{\gamma}$	0,31 ±0,01$^{\beta}$
EEMP 300 mg/kg	97,8 ±2,92$^{\gamma}$	94,4 ±1,63$^{b\gamma}$	47,8 ±1,39$^{\gamma}$	27 ±2,71$^{\gamma}$	0,31 ±0,02$^{\beta}$
MET 20 mg/kg	93,6 ±2,46$^{\gamma}$	93,4 ±2,06$^{\gamma}$	46 ±1,45$^{\gamma}$	28,7 ±3,56$^{\gamma}$	0,31 ±0,01$^{\beta}$

Results are presented as mean ± SEM (n = 5). $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$: significant difference compared with the normal control group. $^{\alpha}P<0.05$, $^{\beta}P<0.01$, $^{\gamma}P<0.001$: significant difference compared with the diabetic control group. NC: normal control; DC: diabetic control; EEMP: Ethanolic Extract of Meiganga Propolis; MET: metformin.

3.6. Effects of EEMP on Liver and Kidney Parameters in Rats Fed by Hypercaloric Sucrose Diet

Table 4 shows the effects of EEMP on selected hepatic and renal parameters and total protein levels. The table shows that the combined administration of the HSD and distilled water resulted in a significant ($p<0.001$) increase in ALAT, creatinine and urea levels in rats in the diabetic control group compared with rats in the normal control group. However, the combination of this food with either EEMP or metformin prevented a significant increase in these same parameters. The value of this enzyme in the blood fell from 112 ± 2.67 U/L in rats in the diabetic control group to 78.6 ± 1.12 and 73 ± 1.48 U/L respectively in rats treated with EEMP 300 mg/kg and metformin 20 mg/kg.

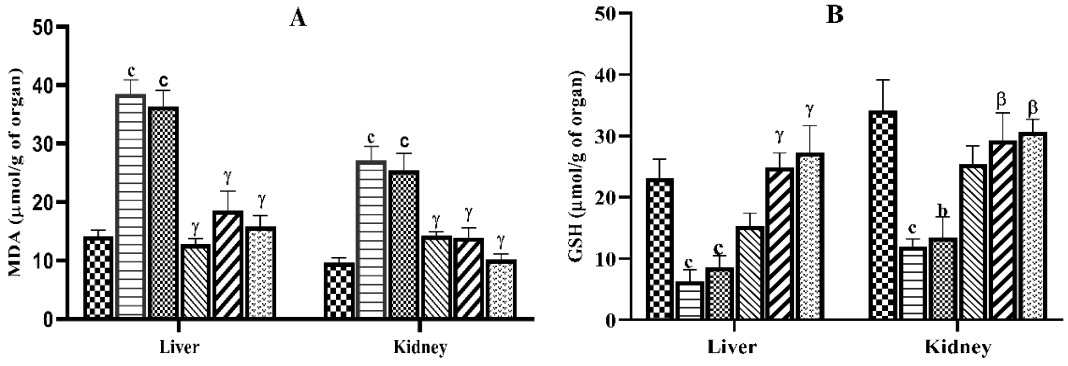
Table 4. *Effects of EEMP on liver and kidney parameters in rats fed by hypercaloric sucrose diet.*

Groupes	ALAT (U/L)	ASAT (U/L)	Créa (mg/dL)	Urée (mg/dL)	Bil (µmol/L)	TP (g/dL)
NC	83,4 ±5,31	68,2 ±3,73	0,5 ±0,12	40 ±4,17	0,43 ±0,08	9,92 ±2,79
DC	112 ±2,67^{c}	68 ±2,59	1,18 ±0,16^{c}	62,8 ±4,18^{c}	0,63 ±0,1	8,2 ±0,97
EEMP 75 mg/kg	103,7 ±3,4^{b}	69,5 ±2,23	1,05 ±0,23^{c}	56,8 ±3,42^{a}	0,6 ±0,5	7,9 ±0,91
EEMP 150 mg/kg	97,8 ±6,45$^{\gamma}$	64,8 ±1,66	0,84 ±0,11	53 ±1,7^{b}	0,56 ±0,07	8,2 ±1,71
EEMP 300 mg/kg	78,6 ±1,12$^{\gamma}$	59 ±2,35	0,47 ±0,009$^{\gamma}$	51,2 ±1,24$^{a\alpha}$	0,54 ±0,09	9,6 ±0,93
MET 20 mg/kg	73 ±1,48$^{\gamma}$	69,6 ±0,6	0,44 ±0,02$^{\gamma}$	40,8 ±1,36$^{\gamma}$	0,54 ±0,05	9,40 ±0,93

Results are presented as mean ± SEM (n = 5). $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$: significant difference compared with the normal control group. $^{\alpha}P<0.05$, $^{\beta}P<0.01$, $^{\gamma}P<0.001$: significant difference compared with the diabetic control group. NC: normal control; DC: diabetic control; EEMP: Ethanolic Extract of Meiganga Propolis; MET: metformin.

3.7. Effects of EEMP on Oxidative Stress Parameters in Rats Fed a Hypercaloric Sucrose Diet

The effect of EEMP on the levels of MDA (A), GSH (B), CAT (C), SOD (D) AND NO (E) measured in the liver and kidneys is presented in Figure 4. The combined administration of the HSD and distilled water resulted in a significant ($p<0.001$) increase in MDA levels in animals from the diabetic control group compared with normal rats but significant ($p<0.001$) decrease in levels of GSH, CAT, SOD and NO. Treatment of the animals with either EEMP or metformin for 30 days prevented a significant ($p<0.001$) increase in this MDA level and decrease in levels of GSH, CAT, SOD and NO compared to the normal control group. These values for MDA were 18.56 ± 3.29 and 15.8 ± 1.91 µmol/g of organ in the liver, 13.95 ± 1.63 and 10.23 ± 0.87 µmol/g of organ in the kidney. For GSH 24.83 ± 2.39 and 27.33 ± 4.34 µmol/g of organ in the liver; 29.24 ± 4.51 and 30.69 ± 2.03 µmol/g in the kidney, for SOD 81.72 ± 2.12 and 70.83 ± 1,69 µmol/g of organ in the liver; 82.8 ± 4.9 and 64.8 ± 3.6 µmol/g of organ in the kidney, for CAT 9.17 ± 0.45 and 7.97 ± 0.97 µmol/g of organ in the liver; 10.28 ± 2.15 and 7.46 ± 0.16 µmol/g of organ in the kidney and finally for NO of 19.71 ± 5.18 and 27.33 ± 9.7 µmol/g of organ in the liver; 25.64 ± 2.44 and 22.1 ± 5.31 µmol/g in the kidney respectively for EEMP 300 mg/kg and metformin 20 mg/kg.



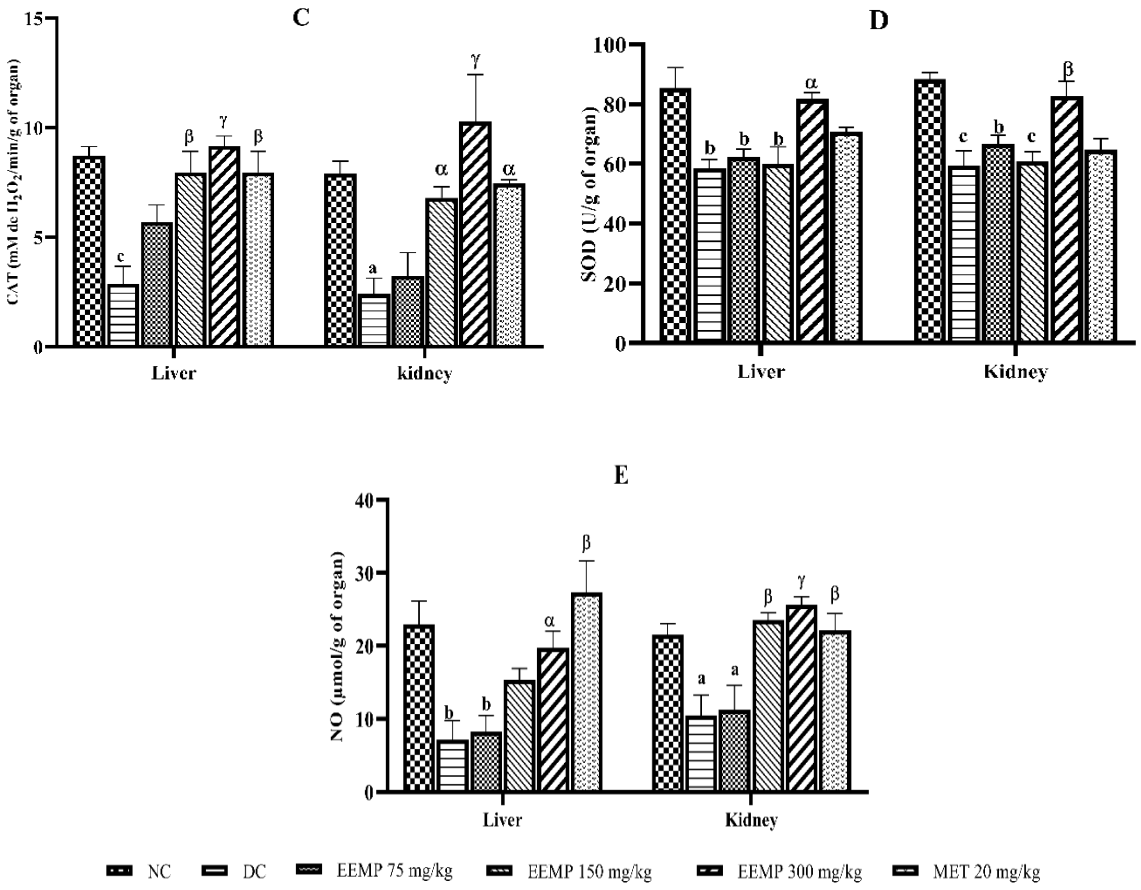


Figure 4. *Effects of EEPM on oxidative stress parameters (MDA (A), GSH (B), CAT (C), SOD (D) and NO (E)) in rats fed a hypercaloric sucrose diet.*

Each bar represents the mean of each group ± SEM (n = 5). [a]P<0.05; [b]P<0.01; [c]P<0.001: significant difference compared with the normal control group. [α]P<0.05; [β]P<0.01; [γ]P<0.001: significant difference compared with the diabetic control group. NC: normal control; DC: diabetic control; EEMP: Ethanolic Extract of Meiganga Propolis; MET: metformin.

4. Discussion

Taking into consideration the epidemiological, etiological and risk factor aspects of human T2DM, it is important to choose an animal model that can mimic the real physiological state for testing the antidiabetic phytomedicines [37]. A characteristic factor of this model should be the onset of insulin resistance. Studies have reported that rats fed HSD develop insulin resistance more easily [28]. Dexamethasone is generally used because of its ability to promote the development of insulin resistance by decreasing the number and activity of glucose transporters [38]. Consequently, the animal model of type 2 diabetes induced by a hypercaloric sucrose diet coupled with a daily injection of 0.2 mg/kg dexamethasone is the one that could mimic the metabolic characteristics of type 2 diabetes in humans. This model was chosen in the present study to assess the effects of the EEMP.

The results obtained show that DHS combined with dexamethasone causes hyperglycaemia, dyslipidaemia and a remarkable reduction in the body weight of the animals. The results of the oral glucose tolerance test (OGTT) suggest that EEMP has an antihyperglycaemic effect at doses of 150 and 300 mg/kg which is similar to that of the reference drug metformin (20 mg/kg). This test suggests that EEMP acts either by slowing intestinal glucose absorption or by stimulating insulin secretion, or that this extract acts by inhibiting glucagon and/or alpha-glucosidase secretion [39].

The fasting blood glucose levels of the animals following administration of the EEMP fell from the tenth day of induction until the end of induction (day 30). This reduction could be due to the presence of certain chemical compounds in the EEMP which phytochemical analysis has revealed to be present (polyphenols, alkaloids, flavonoids, tannins and terpenoids) with powerful antidiabetic properties [40, 41]. Terpenoids are recognized for their ability to reduce glycaemia by a number of mechanisms, including peripheral consumption of glucose in the muscles, and inhibition of gluconeogenesis and glycogenolysis [42, 43]. Flavonoids and polyphenols are able to lower blood glucose levels by enhancing GLUT-2 expression in pancreatic β-cells and in-

creasing GLUT-4 expression and translocation promotion, as well as by inhibiting α-glucosidase [44, 45]. Saponins play a role in reducing glycaemia by inhibiting intestinal glucose absorption and increasing glucose storage, thereby increasing insulin secretion [46].

Insulin resistance inhibits cellular glucose transport, leading to increased catabolism of myofibrillar proteins, inhibition of amino acid uptake and reduced protein synthesis, resulting in hypoproteinemia which accentuates muscle wasting and loss of body mass [47]. In our study, all diabetic control rats showed a loss of body weight over the course of the experiment although there was no change in relative organ mass. However, the animals given EEMP at 300 mg/kg showed a slight increase in body weight in the last two weeks of the experiment. This result testifies to the protective effect of the extract against muscle wasting, which is probably due to the presence of flavonoids and saponins, which stimulate amino acid capture and protein synthesis, as well as inhibiting proteolysis, allowing glucose to be used as an energy source [48].

Dyslipidemia characterized by high levels of total cholesterol, triglyceride and LDL-c followed by low levels of HDL-c was observed in animals in the diabetic control group. Simultaneous administration of EEMP with a hypercaloric sucrose feed prevented the dyslipidemia observed in the diabetic control group. According to Nkono *et al*. (2014), inhibition of lipoprotein lipase (LPL) activity prevents the hydrolysis of triglycerides into glycerol and fatty acids, thereby protecting the body against dyslipidemia. The protective or preventive effect observed with our extract would therefore be due to its ability to inhibit the activity of lipoprotein lipase (LPL) to prevent the hydrolysis of triglycerides into glycerol and fatty acids [49]. Administration of EEMP with HSD prevents an increase in the atherogenic index, suggesting the existence of antidyslipidaemic properties in our extract. These results are in line with those of Orsolic *et al*. (2012); El-Sayed *et al.* (2011) and Zhu *et al*. (2009) who showed that propolis extracts reduce total cholesterol and triglyceride levels in the blood, thereby reducing the risk factors for cardiovascular disease [50-52].

The results show that DHS and dexamethasone administration induces a significant increase in ALAT levels, which is generally considered to be a marker of hepatocellular injury. There was also a significant increase in creatinine and urea levels compared with diabetic control animals. Treatment with EEMP significantly prevented increases in ALAT, creatinine and urea, suggesting a potential protective effect of the extract on liver and kidney function. The presence of flavonoids and saponins in an extract is thought to be responsible for the protective effect on the liver and kidneys as shown by Ajayi *et al.* (2011) and Tran *et al.* (2001) [53, 54]. In addition to flavonoids, our propolis extract also contains saponins, so this protective effect on liver and kidney function is due to the presence of these bioactive compounds in our extract. This extract could act by facilitating the reduction or inhibition of amino acid catabolism.

Oxidative stress contributes directly to the formation of free radicals and therefore to the complications of diabetes [55]. Antioxidants are able to resist oxidative stress by scavenging free radicals, inhibiting lipid peroxidation and increasing the activity of GSH, SOD, CAT and NO [56]. The combined administration of the sucrose hypercaloric food and the EEMP prevents an increase in MDA levels, while increasing the activities of GSH, SOD, CAT and NO. This extract is thought to act either by improving insulin sensitivity, stimulating NOS synthesis, inactivating the NOS inhibitor, or by ensuring the availability of L-arginine and/or reducing oxidative stress [48]. According to Rivera-Yanez *et al*. (2018), polyphenols and flavonoids are potential antioxidants and very good metal chelators. Our extract being administered in combination with a high-calorie food would thus prevent the formation of free radicals and consequently also prevent diabetic complications [57].

5. Conclusion

Type 2 diabetes is widespread throughout the world, but current therapies for this disease using oral anti-diabetic agents have numerous undesirable effects. The results of the present study allow us to conclude that the EEMP at a dose of 300 mg/kg had a preventive action by preventing the increase in fasting blood glucose levels in rats, with a value of 78.25 ± 2.29 mg/dL on day 30. At the same dose, EEMP also prevented an increase in serum LDL-c, ALT and creatinine, with successive values of 27 ±2.71 mg/dL, 78.6 ±1.12U/L and 0.47 ±0.009 mg/dL. However, EEMP 300 mg/kg caused an increase in serum HDL-c, hepatic SOD and CAT, with values of 47.8 ±1.39 mg/dL, 81.72 ±2.12 µmol/g organ and 9.17 ±0.45 µmol/g organ respectively. This study justifies the use of the Ethanolic Extract of Meiganga Propolis in alternative medicine in Cameroon. In the future, we envisage studying the effect of EEMP on intestinal and muscular glucose uptake.

Conflicts of Interest

The authors declare no conflicts of interest.

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