World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

In vivo evaluation of wound healing effect and antioxidant properties of extracts and fractions of *Gymnema sylvestre*

Barnabé Lucien Nkono Ya Nkono [1,*], Samuel Arsène Ntyam Mendo [1], Balthazar Tcheudi Tchouanka [2], Lionnel Jospin Ganno Zébazé [1], Georges Ramone Adinga Abéga [2], Roussel Chatelain Djougue Alemafack [2] and Frida Longo [1]

[1] *Department of Biological Sciences, Higher Teacher Training College, University of Yaoundé 1, Cameroon.*
[2] *Department of Animal Biology and Physiology, Faculty of Sciences, University of Yaoundé 1, Cameroon.*

World Journal of Advanced Research and Reviews, 2022, 15(02), 232–243

Publication history: Received on 22 June 2022; revised on 28 July 2022; accepted on 30 July 2022

Article DOI: https://doi.org/10.30574/wjarr.2022.15.2.0774

Abstract

Among the causes of mortality, wound healing remains a major problem that often results from imbalance between prooxidants and endogenous antioxidants in diabetics. The aim of this study was to evaluate the wound healing activity of *Gymnema sylvestre* in normoglycemic rat, and its antioxidant properties in type 2 diabetic rat.

Evaluation of the healing properties of extracts (aqueous and methanol) and fractions (methylene chloride and methanol) *G. sylvestre* (10% of fraction or extract) were conducted in female normoglycemic rats and 1% fluoxetine was considered positive control using glycerin as a vehicle, while antioxidant assessments were conducted in male diabetic rats.

Type 2 diabetes was induced in male rats by a high sucrose diet for 12 weeks followed by daily intraperitoneal injection of dexamethasone (8 mg/kg) for 5 consecutive days. Animals with a blood glucose above or equal to 140 mg/kg after 12 hours of fasting were considered diabetic. For the evaluation of wound healing 5 groups consisting of 3 female rats each were formed. Fluoxetine (1%), aqueous (Aq) and methanol (MeOH) extracts as well as methylene chloride (F1) and methanol (F2) fractions of the plant were administered at 10% via utopian application on wounds of about 2.5 cm in diameter. For the estimation of oxidative stress parameters in the diabetic rat, 7 groups of 5 animals each were formed, with three control groups including two negative control groups (normoglycemic and diabetic) receiving the vehicle orally (DMSO 3%) and a positive control group receiving metformin (Met, 200 mg/kg). The four experimental groups were treated orally with administration of Aq (100 mg/kg), MeOH, F1 and F2 (7.5 mg/kg). The different treatments were administered once a day for 14 consecutive days.

Results showed that *G. sylvestre* promoted wound healing ($P>0.05$) in the normoglycemic rat with a stronger effect for F2 and MeOH compared to the untreated group. In the diabetic rat, extracts and fractions of *G. sylvestre* significantly reduced ($P<0.001$) the MDA level, while F1 significantly increased ($P<0.01$) the activity of catalase (CAT) and reduced glutathione (GSH) compared to the untreated diabetes group on day one. These results revealed that *G. sylvestre* exhibited strong wound healing effects and justify the use of this in traditional pharmacopoeia.

Keywords: *Gymnema sylvestre*; Wound healing; Antioxidant; Extracts and fractions; Type 2 diabetes mellitus

* Corresponding author: Nkono Ya Nkono Barnabé Lucien
Department of Biological Sciences, Higher Teacher Training College, University of Yaoundé 1, Cameroon.

1. Introduction

Diabetes mellitus is a chronic disease that causes the death of thousands of people every year around the world, it is a metabolic disorder characterized by chronic hyperglycemia due to reduced insulin secretion or insulin action or both [1]. Diabetes is a real public health threat globally and according to estimates by the International Diabetes Federation in 2019, 463 million people aged 20 to 79 are living with diabetes, or 9.3% of the world's population. 700 million people are expected to develop diabetes by 2045, or 10.9% of the world's population [2]. Diabetes causes numerous complications in the body that can significantly increase the number of premature deaths [3]. Patients with diabetes have an increased risk of developing cardiovascular disease, which is one of the leading causes of death. It has been recognized that one of the factors linking diabetes and cardiovascular disease is oxidative stress which reflects an imbalance between the production of free radicals and antioxidants in favor of the former [4] there are other complications such as diabetic foot which is characterized by a wound healing defect in diabetic patients [5]. In addition, diabetes is involved in the death of more than 4 million people worldwide with nearly 10% in Africa [2].

Several plants have proven their effectiveness in managing diabetes and its complications in the traditional pharmacopeia as in experimental research, among which *Gymnema sylvestre* showed antioxidant activity in the streptozotocin-induced diabetic rat (type 1 diabetes mellitus) [6].

Since the diabetic foot is a major complication in diabetes given that more than 80% of diabetics are type 2. It has been interesting to evaluate the properties of extracts and fractions of *G. sylvestre* on wound healing and also to evaluate its activity on parameters of oxidative stress in order to determine if this plant can be used in any type of diabetes mellitus.

2. Material and methods

2.1. Chemicals and drugs

All chemical were from analytical grade: methanol, methylene chloride and dimethyl sulfoxide (DMSO) were purchased from Sigma Aldrich, Germany. Catalase (CAT), reduced glutathione (GSH), superoxide dismutase (SOD) and malondialdehyde (MDA) were obtained from Labkit, France. Dexamethasone sodium was purchased to Rotexmedica Panpharma, Germany.

2.2. Collection and preparation of plant materials

The whole plant of *G. sylvestre* was obtained at the food market from a traditional therapist. After harvest, the plant was identified to the National Herbarium of Cameroon in comparison to sample N°36306/NHC with synonym *Marsdenia sylvestris*. After identification, the whole plant was cut, dried and then crushed with an electric mill in order to obtain a powder that was kept at room temperature in a dark and perfectly enclosed glass jar. This powder was used to prepare extracts and fractions of *G. sylvestre*.

2.3. Extraction and fractionation of plant sample

2.3.1. Aqueous extract

The preparation of the aqueous extract was done by decoction. In a 1-litre volumetric flask, 250 g of *G. sylvestre* powder was introduced and distilled water was completed to the gauge line. The whole solution was then boiled for 15 minutes and cooled to room temperature. After cooling, the mixture was filtered using Wattman No.4 paper. The resulting filtrate was concentrated in the oven at 45°C and an extraction yield of 10.64% was obtained.

2.3.2. Ethanol extract

The methanol extract of *G. sylvestre* (MetOH) was prepared by maceration. In a 1-litre volumetric flask, 200 g of *G. sylvestre* powder was introduced and methanol was added to the gauge line. The mixture was left at room temperature for 2 days and filtered using Wattman Filter Paper No.4. The filtrate was exhausted in a 20°C oven and an extraction efficiency of 12.33% was obtained.

2.3.3. Fractionation of plant sample

The preparation of the methylene chloride (F1) and methanol (F2) fractions of *G. sylvestre* was done by phase chromatography from the residues obtained during the preparation of the aqueous extract. 100 g of residues were inserted separately into two separating funnels. The aqueous phase obtained after settling was respectively mixed with

methylene chloride and methanol as solvent according to the ampoules and left at rest for a new settling. The phases obtained were then concentrated in the oven at 20°C and the extraction yields of 19.71 and 14.67% were obtained respectively for F1 and F2.

2.4. Animals

The experimental animals used were male and female Wistar albino rats aged 22±2 weeks old at the beginning of the experiment. Male rats were used to evaluate antioxidant properties while female rats were used to evaluate the healing properties of extracts and fraction of *G. sylvestre*.

2.4.1. Breeding

The rats were raised in the pet store at room temperature in polypropylene cages covered with stainless steel mesh and lined with white wood chip litter that was renewed every three days. Animals had free access to water and food with a standard composition with 14% lipid, 60% carbohydrate and 23% protein.

2.4.2. Induction of type 2 diabetes

Diabetes induction was only performed in male rats in two stages. The first induction stage was carried out by subjecting the animals to a high sucrose diet for 12 weeks during which the standard diet was replaced by a high sucrose diet composed of 13 % lipids, 64% carbohydrates and 20% protein. Throughout induction, animals had free access to food and water (20% sucrose solution). The second stage of diabetes induction was done by intraperitoneal injection of dexamethasone (8 mg/kg) once daily for 5 consecutive days [7]. At the end of the induction period, the animals were subjected to a minimum feed fasting of 12 hours. The blood glucose in each animal was then taken by placing a drop of blood on the Accu-Answer® blood glucose strip after the distal end of the tail was cut. Animals with blood glucose above or equal to 140 mg/dl were selected for testing.

2.4.3. Distribution of experimental animals

Evaluation of the antioxidant activity of extracts and fractions of *Gymnema sylvestre* in diabetic rats

The selection of extracts (Aq and MeOH) and fractions (F1 and F2) doses were guided by previous pharmacological studies conducted on *G. sylvestre* as well as the traditionally administered doses [8], [9]. In this study, the different treatments were administered orally for 14 consecutive days. The animals were divided into 7 groups of 5 animals each, as follows:

- Group 1: normoglycemic negative control (NNC, DMSO 3%);
- Group 2: diabetic negative control (DNC, DMSO 3%);
- Group 3: aqueous extract of *G. sylvestre* (Aq, 100mg/kg);
- Group 4: methanol extract of *G. sylvestre* (MeOH, 7.5 mg/kg);
- Group 5: methylene chloride fraction of *G. sylvestre* (F1, 7.5 mg/kg);
- Group 6: methanol fraction of *G. sylvestre* (F2, 7.5 mg/kg);
- Group 7: positive control, metformin (200 mg/kg).

Estimation of wound healing activity

For the evaluation of the properties of extracts and fractions of *G. sylvestre* on wound healing, 3 rats per group were shaved at the back using an electric clipper and after anaesthetized with ether, an average diameter injury of 2.5 cm was made at the back of each animal. The treatment was carried out by utopian application of extracts and fractions on the wound of each animal according to groups and this was repeated once a day during the entire treatment period which lasted 14 days. Fluoxetine (1%) was administered as a positive control and glycerin was used as a vehicle to dissolve the different products to be administered. Animal groups were distributed as follows:

- Groups 1 - 4: extracts and fractions of 10% *G. sylvestre* (Aq, MeOH, F1 and F2 respectively);
- Group 5: fluoxetine at 1%.

2.4.4. Animal sacrifice

Only experimental rats made diabetic were sacrificed on day 14. The blood of the different groups was collected on the first day after anaesthesia with ether by the retro-orbital method using capillary tubes, and at the end of the treatment after anaesthesia and sacrifice of animals in order to carry out the serum dosages. The blood was immediately

centrifuged at 4500 revolutions per minute for 15 minutes and the serum was taken and aliquots were formed and stored in freezing at -20°C for subsequent dosages.

2.5. Experimentations

The animals were treated for 14 days. For the evaluation of oxidative stress, diabetic male rats were given oral gavage once daily between 07:00 and 08:00 in the morning various treatments, while for the assessment of the healing activity of *G. sylvestre*, female rats received different treatments by the utopian application once a day in the same time interval.

2.5.1. Estimation of antioxidant activity

The oxidative stress parameters were determined after centrifugation of the blood collected on days 1 and 14. Endpoints evaluated were endogenous antioxidants (CAT, GSH and SOD) and pro-oxidant MDA.

Determination of catalase activity (CAT)

The determination of catalase activity was carried out according to the method described by Sinha [10]. The calibration curve was obtained by matching the absorbance of the standard tubes to the concentrations of hydrogen peroxide (Y=0.0032X; R2=0.9989) while the determination of catalase activity was calculated using the formula given in Equation No.1.

$$\text{CAT activity} = \frac{\Delta OD}{a \times t \times m} \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Catalase activity in mM of H_2O_2/min/g organs; ΔOD = test OD - white OD; a = slope of calibration curve (0.0038); t = duration of reaction (1 minute); m = mass of organ (g).

Reduced glutathione assay (GSH)

The determination of reduced glutathione was carried out according to the method described by Ellman [11] and the reduced glutathione concentration (GSH) in the sample was determined from equation No.2 after reading the optical densities against the blank at 412 nm.

$$[GSH] = \frac{\Delta OD}{\varepsilon \times L \times m} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

[GSH]= GSH concentration (mol/g organs); ΔOD = OD-test OD white; L = Optical path (1 cm); ε = Molar extinction coefficient (13600/mol/cm); m = Organ mass (g).

Determination of superoxide dismutase (SOD)

The method described by Misha & Fridovish [12] was used to perform SOD assay in the sample. The sample absorbance was measured at 20 and 80 seconds and read against white at the 480 nm wavelength. The determination of the SOD activity was determined by calculation using formulas No.3 and 4 respectively.

$$\Delta A \text{ (min)} = A20S - A80S \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

$$\% \text{ Inibition} = 100 - \left(\frac{\Delta A \text{ test}}{\Delta A \text{ blank}}\right) X\ 100 = \ n\ units\ of\ SOD \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Specific activity of SOD = number of units of SOD/ml/g of protein, ΔA: change in absorbance; A_{20S} = Absorbance measured at 20 seconds; A_{80S} = Absorbance measured at 80 seconds.

Determination of malondialdehyde (MDA)

The MDA assay in the sample was carried out according to the method described by Dahle & Holman [13]. After reading the optical densities at 530 nm against white, the MDA concentration was determined using equation No.5.

$$[MDA] = \frac{\Delta OD}{\varepsilon \times L \times m} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

[MDA]= MDA concentration (mol/g organs); ΔOD = OD test-OD white; L= optical path (1 cm); ε= Molar extinction coefficient (15600/mol/cm); m= mass of the organ (g).

2.6. Estimation of wound healing activity

The healing percentage of the wounds was calculated from the calculation of the healing surface of the corresponding day and the surface of the initial wound, this after the measurement of the healing diameter [14], [15]. Formula No.6 was used to calculate the healing percentage of the wound.

$$\text{Wound healing (\%)} = \frac{(\text{wound surface on day 1} - \text{wound surface on specific day})}{\text{wound surface on day 1}} X100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

2.7. Statistical analysis

Analysis were performed using GraphPad Prism (version 5.0.3). Values were expressed as mean ± standard error of the mean (SEM). Analysis of variance (ANOVA, Bonferroni post-test) and pair t-test were done as the test of significance. The confidence interval was set at 95% with a significance threshold of less than 5% ($P<0.05$).

3. Results

3.1. Effect of *Gymnema sylvestre* wound healing activity in normoglycemic rats.

Figure 1 shows the results of the wound healing process induced by extracts and fractions of *G. sylvestre* in the type 2 diabetic rat. From this figure, the healing diameter decreases in all groups with a greater the last day of testing in animals receiving the F2 fraction, respectively, methanol and aqueous extracts, and finally the F1 fraction.

It should be noted that although the difference in the percentage of wound healing in the groups of animals that received the extracts and fractions of 10% *G. sylvestre* is not significant ($P>0.05$) compared to the positive control group that received 1% fluoxetine, the methanol fraction and methanol extract had a healing percentage of the wound (87.34±3.06% and 87.04±1.85 respectively for F2 and MeOH) higher than that of fluoxetine (86.29±4.83%) (Table 1). Table 1 also shows that on the third day of treatment, the methanol extract had the highest healing percentage of the wound (41.22±2.84%) followed by the methanol fraction (33.18±2.73%), the aqueous extract (30.06±3.08%), the fluoxetine (29.90±3.25%) and the methylene chloride fraction (29.56±3.05%).

3.2. Effect of antioxidant activity of extracts and fractions of *Gymnema sylvestre* in type 2 diabetic rats

The effects of extracts and fractions of *G. sylvestre* on the oxidative status of diabetic rats induced by high dietary sucrose and dexamethasone were evaluated by measuring the activity of a prooxidant (malondialdehyde) and antioxidants (catalase, reduced glutathione and superoxide dismutase).

3.2.1. Effect of Gymnema sylvestre extracts and fractions on catalase activity in type 2 diabetic rats

The results presented in Figure 2 show a significant decrease ($P<0.01$) in catalase activity (mM/ (min x mg protein) in the DNC group on day 1 (41.24±3.05) compared to the NNC group (65.90±3.27). In contrast, in groups treated with extracts and fractions of *G. sylvestre*, there was an increase in catalase activity close to that of normoglycemic animals in the NNC group ($P>0.05$). Despite this increase in activity in the Aq (61.37±4.13), MeOH (51.95±3.36) and F2 (53.30±3.68) groups, there was no significant difference from the DNC group on Day 1, however, there was a significant increase ($P<0.01$) Catalase activity in F1-treated groups (69.42±5.20) and oral metformin antidiabetic (67.17±3.92).

3.2.2. Effect of Gymnema sylvestre extracts and fractions on reduced glutathione activity in type 2 diabetic rats

The effects of extracts and fractions of *G. sylvestre* on reduced glutathione activity (13600 mol/mg protein) are shown in Figure 3. These results indicate a very significant decrease ($P<0.001$) in GSH activity in DNC groups on day 1 (191.70±12.02) and 14 (179.81±7.60) compared to the NNC group (384.11 23.34). For the groups treated with extracts Aq (292.56±19.82) and MeOH (228.57±32.70) as well as fraction F2 (200.10±16.66) and oral antidiabetic metformin (197.38±21.39), although the activity of GSH increased, it is not significantly different ($P>0.05$) from the DNC group on day 1 and 14. In addition, the activity of the GSH groups MeOH ($P<0.01$), F2 ($P<0.001$) and metformin ($P<0.001$) remains significantly low compared to that of the NNC group. On the other hand, in the F1 group (338.33±23.51), GSH activity increased significantly ($P<0.01$) compared to the DNC group on day 1 and 14, so that it no longer shows any significant difference with the NNC group ($P>0.05$).

Table 1 Effect of extracts and fractions of *Gymnema sylvestre* on wound healing in normoglycemic rats

Time (Day)	Wound healing percentage (%)					Wound healing surface (mm^2)				
	Fluoxetine (1%)	**Aq (10%)**	**MeOH (10%)**	**F1 (10%)**	**F2 (10%)**	**Fluoxetine (1%)**	**Aq (10%)**	**MeOH (10%)**	**F1 (10%)**	**F2 (10%)**
1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	271.08±40.86	273.57±18.25	278.65±33.34	255.51±40.51	279.27±26.83
3	29.90±3.25	30.06±3.08	41.22±2.84	29.56±3.05	33.18±2.73	184.06±29.14	161.48±16.44	195.04±19.89	165.56±14.72	194.02±8.99
6	34.56±2.82	44.54±4.54	43.58±1.26	31.92±2.34	39.41±3.45	145.67±17.55	154.75±13.43	187.50±15.34	148.64±15.78	181.25±13.63
9	61.23±1.15	57.75±3.41	63.26±2.91	47.57±4.89*	46.39±2.06**	109.54±20.33	101.52±14.34	141.41±23.32	135.54±17.28	108.18±10.28
12	80.21±5.12	71.20±5.21	76.71±0.80	69.65±3.52	81.11±1.60	74.11±12.65	63.46±2.96	78.85±26.87	46.99±4.03	52.76±10.66
14	86.29±4.83	81.54±3.03	87.04±1.85	79.02±3.89	87.34±3.06	48.14±8.67	34.96±3.78	53.28±22.04	30.24±3.39	36.25±11.26

Values are expressed as mean ± ESM (n=3). *P<0.05, **P<0.01: significant differences from the positive control group (fluoxetine). Aq: aqueous extract; MeOH: methanolic extract; F1: methylene chloride fraction; F2: methanolic fraction.

Aq (a, b, c): aqueous extract; MeOH (d, e, f): methanolic extract; F1 (g, h, i): methylene chloride fraction; F2 (j, k, l): methanolic fraction; fluoxetine (m, n, o).

Figure 1 Effect of extracts and fractions of *Gymnema sylvestre* on wound healing in normoglycemic rats

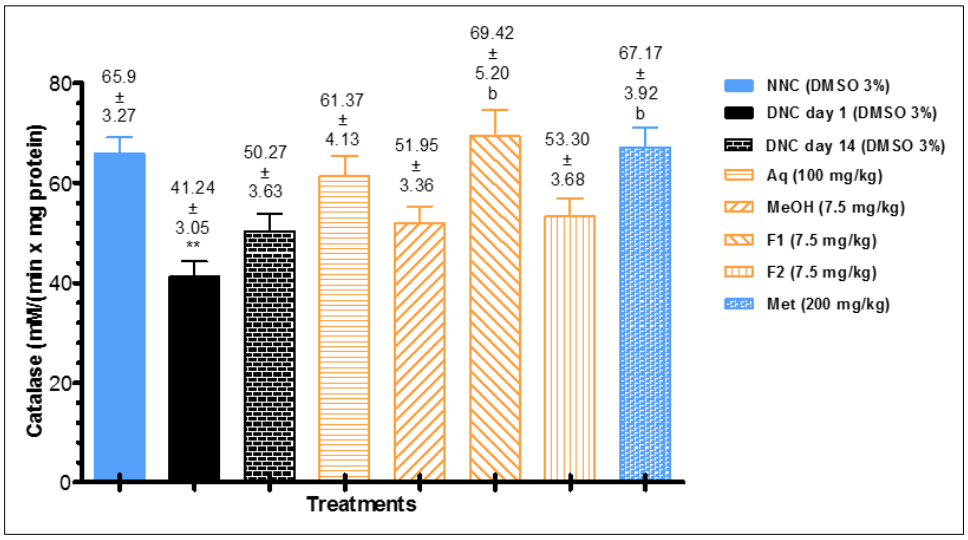

Figure 2 Effect of *Gymnema sylvestre* extracts and fractions on catalase activity in type 2 diabetic rats

Values are expressed as mean ± ESM (n=5). **P<0.01: significant difference from NNC group. ᵇP<0.01: significant difference from DNC group on day 1. NNC: normoglycemic negative control; DNC: diabetic negative control; Aq: aqueous extract; MeOH: methanolic extract; F1: methylene chloride fraction; F2: methanolic fraction; Met: metformin.

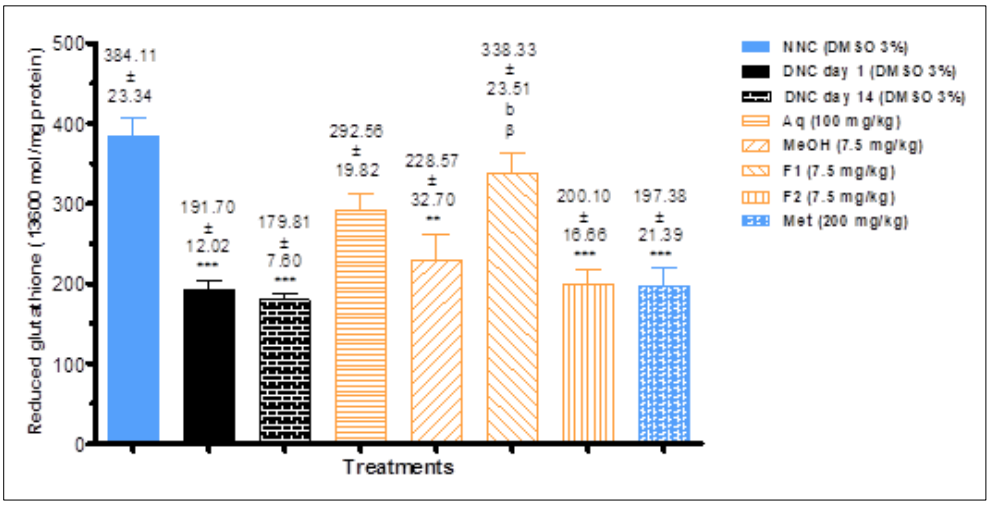

Figure 3 Effects of *Gymnema sylvestre* extracts and fractions on reduced glutathione activity in type 2 diabetic rats

Results are expressed as mean ±ESM (n=5). **P<0.01, ***P<0.001: significant differences from the NNC group. ᵇP<0.01: significant difference from DNC group on day 1; ᵝP<0.01: significant difference from DNC group on day 14. NNC: normoglycemic negative control; DNC: diabetic negative control; Aq: aqueous extract; MeOH: methanolic extract; F1: methylene chloride fraction; F2: methanolic fraction; Met: metformin.

3.2.3. Effect of Gymnema sylvestre extracts and fractions on superoxide dismutase activity in type 2 diabetic rats

The effects of *G. sylvestre* extracts and fractions on superoxide dismutase activity (IU/g protein) are shown in Figure 4. These results indicate a highly significant decrease (P<0.01) in SOD activity in the DNC group on Day 1 (199.55±10.91) and 14 (213.20±14.76) compared to the NNC group (324.69±19.04). These results also reveal that although there was an increase in SOD activity in the groups that received the extracts Aq (263.63±17.39) and those that received the fractions MeOH (286.29±22.26) and fractions groups F1 (221.77±14.72) and F2 (289.56±19.07), it remains non-significant compared to the DNC group on Day 1 and 14. However, in the F1 group, SOD activity remains significantly low (P<0.05) compared to the NNC group. These results also show a significant increase in SOD activity in the metformin-treated group (315.97±18.20), compared to the DNC groups on day 1 (P<0.01) and DNC groups on day 14 (P<0.05).

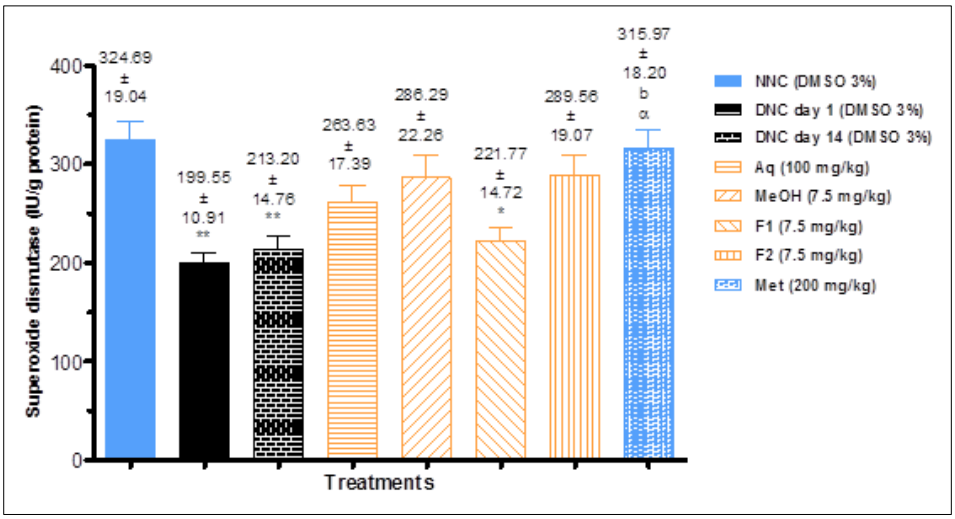

Figure 4 Effect of extracts and fraction of *Gymnema sylvestre* on superoxide dismutase activity in type 2 diabetic rats

Results are expressed as mean ±ESM (n=5). **P<0.01, **P<0.01: significant differences from the NNC group. ^{b}P<0.01: significant difference from DNC group on day 1; $^{\alpha}$ P<0.05: significant difference from DNC group on day 14. NNC: normoglycemic negative control; DNC: diabetic negative control; Aq: aqueous extract; MeOH: methanolic extract; F1: methylene chloride fraction; F2: methanolic fraction; Met: metformin.

3.3. Effect of *Gymnema sylvestre* extracts and fractions on malondialdehyde activity in type 2 diabetic rats.

The effects of *G. sylvestre* extracts and fractions on MDA activity are shown in Figure 5. These results show a highly significant (P<0.001) increase in MDA activity (15600 mol/mg protein) in DNC groups on day 1 (353.23±22.89) and 14 (284.33±17.57) compared to the NNC group (123.63±10.73). Despite the fact that this activity decreased in the DNC group on Day 14, however, it did not show any significant difference (P>0.05) from the DNC group on Day 1. In contrast, MDA activity (15600 mol/mg protein) in the metformin (172.54±12.52), Aq (204.61±12.05) and MeOH (146.43±14.15) groups, and F1 (171.99±12.90) and F2 (182.19±12.24) fractions significantly decreased (P<0.001) compared to DNC on day 1. Similarly, comparison with the DNC group on day 14 shows that MDA activity ((15600 mol/mg protein) decreased significantly in the extract groups (Aq (P<0.05), MeOH (P<0.001)), fractions (F1 (P<0.01), F2 (P<0.01), and metformin (P<0.01). The decrease in MDA activity is very pronounced in the MeOH, F1, F2 and Met groups so that they no longer show significant differences with the NNC group (P>0.05).

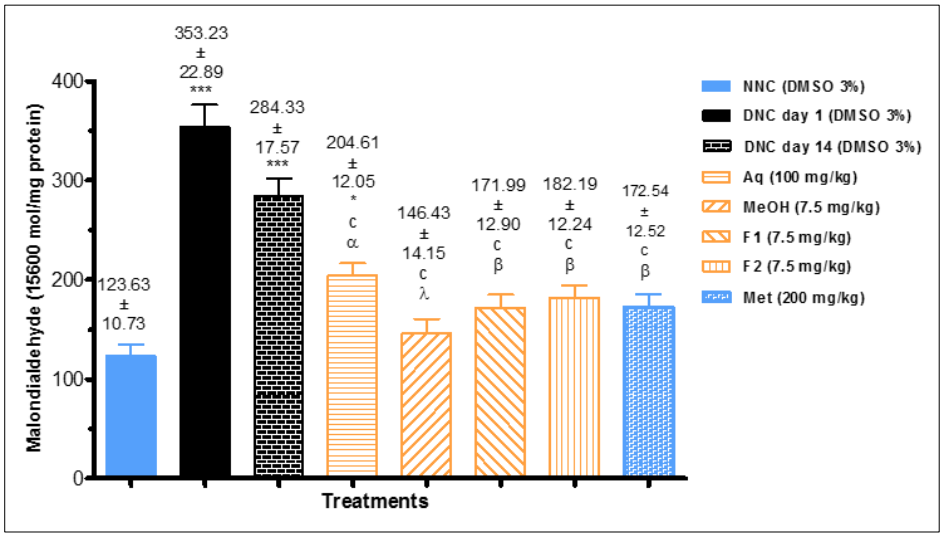

Figure 5 Effect of *Gymnema sylvestre* extracts and fractions on Malondialdehyde activity in type 2 diabetic rats.

Results are expressed as mean ±ESM (n=5). $^{*}P<0.05$, $^{***}P<0.001$: significant differences from the NNC group. $^{c}P<0.001$: significant difference from DNC group on day 1; $^{\alpha}P<0.05$, $^{\beta}P<0.01$, $^{\lambda}P<0.001$: significant differences from DNC group on day 14. NNC: normoglycemic negative control; DNC: diabetic negative control; Aq: aqueous extract; MeOH: methanolic extract; F1: methylene chloride fraction; F2: methanolic fraction; Met: metformin.

4. Discussion

In this study, the activity of *G. sylvestre* on wound healing and antioxidant activity were evaluated respectively in the normoglycemic rat and in the type 2 diabetic rat. Type 2 diabetes mellitus is generally associated with an insulin resistance which, when chronic promotes complications that can affect several organs including the skin. One of the complications related to diabetes is the diabetic foot which is a consequence of peripheral neuropathy, which results in a decrease or loss of the sensitivity of the lower limbs due to nerve damage [16]. The individual suffering from diabetic foot has a scarring defect in the wounds of his lower limbs [2]. In our study, the healing effects of *G. sylvestre* extracts and fractions were evaluated in female normoglycemic rats. These results showed that extracts and fractions of *G. sylvestre* allow a good wound healing. However, the results were more significant with the methanol fraction (10%) and the methanol extract (10%) which had wound healing activity similar to that of fluoxetine (1%). It should be noted that the wound healing activity of the methanol fraction and methanol extract of *G. sylvestre* was slightly higher than that of fluoxetine used in this study as a positive control. Fluoxetine is a drug belonging to the class of serotonin reuptake inhibitors, which is used as a first-line antidepressant [17]. Moreover, the fluoxetine used during this manipulation contained penicillin which has proven its effectiveness in the scar process for several decades [18]. The data obtained in this study indicate that extracts and fractions of *G. sylvestre* could influence wound healing, scarring and fibrosis, on the one hand by preventing proliferation and shrinkage of myofibroblasts, and on the other hand by acting on the metabolism of collagen [19]. Extracts and fractions of *G. sylvestre* could therefore act in the same way as penicillin, given that phytochemical studies of *G. sylvestre* have revealed the presence of saponins and anthraquinones that have known antimicrobial and anti-inflammatory properties [20]. The effects of extracts and fractions of *G. sylvestre* on wound healing could therefore be assimilated to the actions of these compounds.

It is known that chronic hyperglycemia is one of the major causes of the imbalance of the balance between the levels of prooxidants and endogenous antioxidants, manifested by an increased increase in oxygen reactive species and the level of endogenous prooxidants including MDA [4], [6], [21]. Dans notre étude, nous avons observé une augmentation très significative (P<0,001) de l'activité du MDA chez les rats diabétiques au jour 1, en comparaison aux rats normoglycémiques. In the diabetic subject, hyperglycemia is responsible for a decrease in gene expression of certain antioxidant enzymes including CAT and SOD [4]. This was also observed in our study because in comparison with normoglycemic rats, a significant decrease in CAT (P<0.01), SOD (P<0.01) and GSH (P<0.001) activity was observed in diabetic rats on day 1. The increase in endogenous prooxidant MDA activity and the decrease in endogenous antioxidants (CAT, SOD and GSH) would be due to the combined action of the hypercaloric diet and the intraperitoneal injection of dexamethasone previously submitted by the animals.

In this study, after 14 days of administration of extracts and fractions of *G. sylvestre*, a significant reduction in MDA activity was observed compared to the untreated diabetes group, with further reduction in the group of animals receiving methanol extract (P<0.001), followed by fraction F1 (P<0.01), F2 (P<0.01) and aqueous extract (P<0.05) respectively. Our results are thus in agreement with those of El Shafey et al who showed that the aqueous extract of the leaves of *G. sylvestre* decreases the activity of MDA in the diabetic rat type 1 induced with dexamethasone [22].

The results obtained in this study are also in agreement with those of Fatani et al which showed that the aqueous extract of *G. sylvestre* at doses of 50 and 100mg/kg increases the activity of catalase, SOD and GSH in diabetic rats induced by streptozotocin [6]. The antioxidant properties of *G. sylvestre* could be explained by the reduction in the production of reactive oxygen species (ROS) and advanced glycation end products (AGE) on the one hand and on the other hand by the increase in gene expression of antioxidants. Given that after the treatment of diabetic rats with extracts and fractions of *G. sylvestre*, no significant difference (P>0.05) was observed in the CAT and GSH values compared to the normoglycemic control group. In contrast, for SOD, the methylene chloride (F1) fraction significantly increased (P<0.05) the activity of SOD. These results suggest extracts and fractions of *G. sylvestre* boosts and restores antioxidant activity in diabetic rats.

5. Conclusion

This study shows that extracts and fractions of *G. sylvestre* improve the antioxidant status of diabetic rats by decreasing the activity of prooxidant MDA and increasing the activity of endogenous antioxidants such as CAT, GSH and SOD. In

addition, extracts and fractions improved the wound healing process in the normo-glycemic rat with greater action in animals treated with the methanol fraction (F2) and methanol extract (MeOH).

Compliance with ethical standards

Acknowledgments

The authors would like to thank the Ministry of Higher Education of Cameroon for funding this study through the Research Modernization Fund.

Disclosure of conflict of interest

The authors state that there is no conflict of interest.

Statement of ethical approval

All the animal experiments were carried out in accordance with the guidelines of Cameroon National Ethics committee (Ref. FWIRB 00001954).

References

[1] Punthakee Z, Goldenberg R, Katz P. Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome. Can J Diabetes. 2018, 42:S10–5.

[2] International Diabetes Federation (IDF). IDF Diabetes Atlas: Ninth edition 2019. Vol. 266, The Lancet. 2019. 134–137 p.

[3] World Health Organization. World health statistics - monitoring health for the SDGs. World Heal Organ. 2016, 1–121.

[4] Haleng J, Pincemail J, Defraigne JO, Charlier C, Chapelle JP. Oxydantive stress. Rev Med Liege. 2007, 62(10):628–38.

[5] Chalupa LM, Werner JS. The visual neurosciences. Massachusetts Institute of Technology; 2004. 1813 pages.

[6] Fatani AJ, Al-Rejaie SS, Abuohashish HM, Al-Assaf A, Parmar MY, Ola MS, et al. Neuroprotective effects of *Gymnema sylvestre* on streptozotocin-induced diabetic neuropathy in rats. Exp Ther Med. 2015, 9(5):1670–8.

[7] Hemanth Kumar V, Nagendra Nayak IM, Huilgol S V., Yendigeri SM, Narendar K, Rajasekhar CH. Dose dependent hepatic and endothelial changes in rats treated with dexamethasone. J Clin Diagnostic Res. 2015, 9(5):FF08-FF10.

[8] Raji RO, Muhammad HL, Abubakar A, Maikai SS, Raji HF. Acute and sub-acute toxicity profile of crude extract and fractions of *Gymnema sylvestre*. Clin Phytoscience. 2021, 7(56):1–9.

[9] Kumar PM, Venkataranganna M V., Manjunath K, Viswanatha GL, Ashok G. Methanolic leaf extract of *Gymnema sylvestre* augments glucose uptake and ameliorates insulin resistance by upregulating glucose transporter-4, peroxisome proliferator-activated receptor-gamma, adiponectin, and leptin levels in vitro. J Intercult Ethnopharmacol. 2016, 5(2):146–52.

[10] Sinha. Estimation of catalase. Appendices. 1972, 28:189.

[11] Ellman GL. Tissue Sulfhydryl Groups. Arch Biochem Biophys. 1959, 82:70–7.

[12] Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem [Internet]. 1972, 247(10):3170–5. Available from: http://dx.doi.org/10.1016/S0021-9258(19)45228-9

[13] Dahle LK, Hill EG, Holman RT. The thiobarbituric acid reaction and the autoxidations of polyunsaturated fatty acid methyl esters. Arch Biochem Biophys. 1962, 98(2):253–61.

[14] Costa A, Cavalcante G. Evaluating the Use of an Ointment Based on Prosopis juliflora Leaves in the Topical Therapy of Wounds. Adv Res. 2015, 5(4):1–6.

[15] Fetse J, Kyekyeku J, Dueve E, Mensah K. Wound Healing Activity of Total Alkaloidal Extract of the Root Bark of Alstonia boonei (Apocynacea). Br J Pharm Res. 2014, 4(23):2642–52.

[16] Monnier L, Colette C, Owens D. The glycemic triumvirate and diabetic complications: Is the whole greater than the sum of its component parts? Diabetes Res Clin Pract [Internet]. 2012, 95(3):303–11. Available from: http://dx.doi.org/10.1016/j.diabres.2011.10.014

[17] Altamura AC, Moro AR, Percudani M. Clinical Pharmacokinetics of Fluoxetine. Clin Pharmacokinet. 1994, 26(3):201–14.

[18] Pohl R, Hunt TK. Penicillin G and Wound Healing. Arch Surg. 1970, 101(5):610–1.

[19] Rooman R, De Wever B, Moeremans M, Geuens G, Daneels G, Aerts F, et al. Ketanserine mode of action investigations into wound healing and fibrosis. Reprod Nutr Développement. 1989, 29(Suppl. 1):10–10.

[20] Khan F, Sarker MR, Ming LC, Simmonds MSJ. Comprehensive review of phytochemical, pharmacological and clinical potentials of *Gymnema sylvestre*. Pharmacology. 2019, 10(1223):1–19.

[21] Tiwari P, Mishra BN, Sangwan NS. Phytochemical and pharmacological properties of *Gymnema sylvestre*: an important medicinal plant. Biomed Res Int. 2014;2014:830285. doi: 10.1155/2014/830285. Epub 2014 Jan 6. PMID: 24511547; PMCID: PMC3912882.

[22] El Shafey AAM, El-Ezabi MM, Seliem MME, Ouda HHM, Ibrahim DS. Effect of *Gymnema sylvestre* R. Br. leaves extract on certain physiological parameters of diabetic rats. J King Saud Univ - Sci. 2013, 25(2):135–41.