



(RESEARCH ARTICLE)



Extracts and fractions of *Gymnema sylvestre* restore liver and kidney function by regulating tissue and serum transaminases in the type 2 diabetic rat

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Abstract

Diabetes mellitus usually induce kidney and liver complications, characterized by an increase in transaminases activity. The aim of this study was to evaluate the activity of *Gymnema sylvestre* on the variation of tissue and serum transaminases activities in high sucrose diet and dexamethasone-induced diabetic rats. The animals were fed with high sucrose diet and 20% sugar water for 12 weeks then, received an intraperitoneal injection of dexamethasone (8mg/kg) once a day for 5 days between 07 and 08 am. After induction, the insulin sensitivity test was performed and the animals were divided into 7 groups. The experimental groups received extracts (aqueous and methanolic) and fractions (methylene chloride and methanol) of *G. sylvestre* once a day for 14 days. At the end of experiment, the animals were sacrificed and the blood, liver and kidney were collected for evaluation of transaminases and histopathology reduced by the plant. The activity of liver and kidney transaminases of the diabetic negative control (DNC) group on days 1 and 14 had significantly elevated ($P < 0.001$) compared with this of the normoglycemic negative control (NNC) group and the different treated groups. This trend was also observed at the serum level but only with ASAT. Histological analysis revealed hepatic leukocyte infiltrations and mesangial expansions in the urinary space in the DNC group on days 1 and 14. These tissue abnormalities were corrected in groups receiving extracts and fractions of *G. sylvestre*. This study suggests that the extracts and fractions of *G. sylvestre* restore tissue damage in diabetic rats.

Keywords: *Gymnema sylvestre*; Insulin resistance; transaminases; liver diseases; kidney failure

1 Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, hypertriglyceridemia and chronic hypercholesterolemia, which results from either a lack of insulin secretion or its insufficient action, or both [1]. According to the International Diabetes Federation, it visualizes itself as a disorder of the assimilation, use and storage of sugars brought by the diet and results in a permanent high level of glucose (≥ 126 mg/dl) in the blood [2]. In 2017, diabetes killed 4 million people worldwide, including 298,160 in Africa. The International Diabetes Federation has estimated 463 million people worldwide, with people living with diabetes in 2019 [3]. According to WHO, diabetes mellitus is no longer a rarity in Africa: depending on the region, the prevalence varies from 1% to 20% [4]. In Cameroon, the prevalence of diabetes among people aged 20 to 79 years continues to increase over the years, from 3.9% in 2010 to 6% in 2019 [5]. Diabetes mellitus is the fifth leading cause of death in Cameroon and kills about 2% of the population each year. It is therefore obvious that this is a condition associated with a significant mortality rate. However, this mortality is related not only to the hyperglycemia characteristic of diabetes mellitus but also to the complications of diabetes including nephropathy and liver damage [6], [7]. These renal and liver damage are manifested not only by structural but also functional changes in these organs. In the diabetic subject, the rate and activity of certain enzymes

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whose transaminases are increasing at the liver, renal and serum levels, thus testifying to tissue damage [8], [9]. To overcome these harmful complications, it is therefore important to take care of your glycemic status. For a long time, the means used were insulin and oral antidiabetics. However, their action decreases with treatment and they are not always within the reach of the patient, especially in developing countries such as Cameroon [10]. In developing countries, people very often use traditional pharmacopoeia to solve their primary health problems, which is less costly and less harmful than conventional treatments. Several plants have proven their antidiabetic efficacy such as *Moringa oleifera*, *Citrus limon* [11] and *Garcinia kola* [12]. In addition to this effect, other plants have revealed their importance in modulating the activity of transaminases in diabetics. This is particularly the case for *Costus pictus* [13] and *Gymnema montanum* [14]. This is also the case with the aqueous extract of *Gymnema sylvestre* (*Marsdenia sylvestris*) in the Streptozotocin-induced type 1 diabetic rat [15]. However, no scientific studies have revealed the effect of this plant in the type 2 diabetic rat induced by the combination of a particular diet and a chemical substance. The purpose of this study was therefore to evaluate the effect of extracts (aqueous and methanol) of *Gymnema sylvestre*, as well as its fractions (methylene chloride and methanol) on the variation of tissue and serum transaminases in the type 2 diabetic rat induced by hypercaloric feeding and dexamethasone.

2 Material and methods

2.1 Chemicals and drugs

All chemical were from analytical grade: D- (+)-glucose, methanol, methylene chloride and dimethyl sulfoxide (DMSO) were purchased from Sigma Aldrich, Germany. Alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) were obtained from Labkit, France. Dexamethasone sodium phosphate and insulin and metformin were respectively purchased to Rotexmedica Panpharma, Germany; Novo Nordisk, France and Denk Pharma GmbH & Co, Germany.

2.2 Plant collection and extraction

The harvest of *G. sylvestre* was made in a popular market of the city of Yaoundé (Cameroon) with a trader practicing in traditional medicine. The whole plant was obtained in the dry state and the identification was made to the National Herbarium of Cameroon (NHC) in accordance with sample N°36306/NHC. After identification, the plant was ground with an electric mill and then sifted to obtain a fine powder that allowed the preparation of extracts (aqueous and methanol) and fractions (methylene chloride and methanol) of *G. sylvestre*.

2.2.1 Preparation of aqueous extract

The aqueous extract was prepared by decoction by inserting 250 g of powder into a flask and then distilled water was added to obtain a total volume of one litre. The mixture was then brought to a boil for 15min and then left to rest until completely cooled. After cooling, the decoction was filtered using Wattman N°4 filter paper and the resulting filtrate was used up in the oven at 45°C to obtain a dry extract mass of 26.6 g.

2.2.2 Preparation of ethanol extract

The methanol extract was prepared by macerating 200 g of powder into a flask, then ethanol was added to obtain a total volume of one liter, which was left at rest for 48 hours. The mixture was then filtered using Wattman filter paper No 4. The filtrate was exhausted in the oven at 20°C and an extract mass of 24.67 g was obtained.

2.2.3 Preparation of fractions

The methylene chloride (F1) and methanol (F2) fractions were prepared from residues obtained after the aqueous extract was prepared. In two separating funnels, 100 g of extract was introduced and methylene chloride and methanol were each added to a funnel and the mixture of each funnel was agitated and left at rest for settling. The supernatant liquids were then concentrated in the oven at 20°C and the masses of 19.71 g and 14.67 g were obtained for fractions F1 and F2 respectively.

2.3 Animals

The study was conducted in male albino Wistar rats (*Rattus norvegicus*) aged 09±2 weeks at onset of diabetes induction. These animals were reared under ambient temperature conditions with a 12:12 hours light-dark cycle in polypropylene cages containing chips and covered with stainless steel mesh. Animals had free access to water and a standard-composition diet containing 14% lipid, 60% carbohydrate, 23% protein and 3% mineral salts. These animals were treated according to the standards of the National Ethics Committee of Cameroon (Ref. FWIRB 00001954).

2.3.1 Induction of diabetes

The induction of diabetes in rats was done in two phases: the first phase lasted 12 weeks and consisted of the rats' habituation to a high sucrose diet containing 10% fat, 74% carbohydrates, 14% protein and 2% mineral salts). During this phase, the animals had free access to sugar water with 20% sucrose.

After 12 weeks of feed induction, animals were received an intraperitoneal injection of dexamethasone at 8 mg/kg daily for 5 consecutive days [16]. The animals selected for the experiments were those who, 2 days after discontinuation of dexamethasone injection, had fasting blood glucose greater than or equal to 140 mg/dl.

2.3.2 Distribution of animals

After the induction phase, the animals were tested for insulin sensitivity and randomized into 7 groups of 5 animals each qui received the various treatments orally as follows:

- Group 1: normal negative control (NNC), DMSO 3% 10 ml/kg;
- Group 2: diabetic negative control (DNC), DMSO 3% 10 ml/kg;
- Group 3: aqueous extract of *G. sylvestre* (Aq), 100 mg/kg;
- Group 4: methanol extract of *G. sylvestre* (MetOH), 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 received metformin (Met): 200 mg/kg.

2.4 Experimentations

The experiments took place over a period of 14 days. During this period, DMSO, extracts (aqueous and methanol), fractions (methylene chloride and methanol) and metformin were administered once daily between 07:00 and 08:00 in the morning. 3% DMSO was used as a vehicle.

2.4.1 Insulin sensitivity test

This test was performed on day 1 prior to the experiments to reassure that the animals were insulin-resistant. After a 12-hour, waterless fasting, the blood glucose levels of the animals were taken. Based on these fasting blood glucose levels, the induction animals were grouped into 4 groups. Rats in groups 1, 2, 3 and 4 had fasting blood glucose levels of 300 and 360 mg/dl, 200 and 299 mg/dl, 120 and 199 mg/dl, respectively, and less than 120 mg/dl. The different groups of animals were then given an intraperitoneal injection of insulin at 5 IU/kg body weight and blood glucose levels were taken by placing a drop of blood on a test strip of a Accu-Answer® brand blood glucose meter after distal puncture of the tail before insulin injection and then at 10, 20, 60 and 120 minutes respectively after insulin injection.

2.4.2 Evaluation of serum, hepatic and renal transaminases activity

Prior to the experiments, 5 diabetic animals were anesthetized and sedated by intravenous injection of a single dose of ketamine (50 mg/kg) and diazepam (5 mg/kg) respectively and were subsequently sacrificed. The same was true for all animals in the 7 lots on day 14 when the experiments were completed. The blood of each animal was collected and centrifuged at 4500 revolutions per minute for 15 minutes. The liver and kidneys were collected and weighed to determine their relative weights.

Subsequently, 0.2 g of organ was crushed with mortar and pestle and 1.8 ml of NaCl 9‰ was added and the resulting homogenate was centrifuged at 4500 revolutions per minute for 15 minutes. In order to preserve the enzymatic activity, during these manipulations, the samples were kept in ice baths. The homogenates and serums were kept in Eppendorf tubes at -20°C for subsequent ALAT and ASAT assays using the Labkit assay kits (France). The activity of transaminases was calculated according to the following formula:

$$\text{Activity of ALAT or ASAT (U/l)} = A_{\text{sample}} \times 1750$$

Where, A_{sample} = absorbance of sample; 1750 = coefficient given by the Labkit dosing kit.

2.4.3 Effects of extracts and fractions on body weight variation and relative liver and kidney masses

To assess the effect of *G. sylvestre* extracts and fractions on the body mass variation of diabetic rats, individual animal masses were taken at the beginning of the experiment and every 3 days. After sacrificing the animals, the liver and

kidneys were removed and weighed. The relative weight of these organs has been calculated according to the following formula:

$$\text{Relative weight of the organ} = (\text{organ mass} / \text{body weight}) \times 100$$

2.4.4 Histopathological investigation of liver and kidney

After the animals were sacrificed, the large lobe of the liver and the left kidney were used for the histological sections as described previously [17]. The tissues were fixed to 10% formalin and dehydrated. The inclusion in paraffin was subsequently done before the microtome cuts were made. The cuts were then stained with eosin & hematoxylin and were observed at a magnification of 200.

2.4.5 Phytochemical screening of the plant

Phytochemical analysis of the plant was carried out using the method described by Harborne [18]. Thus, the presence or absence of several secondary metabolites has been demonstrated, including tannins, phenols, saponins, flavonoids, alkaloids and coumarin.

2.5 Statistical analysis

Statistical analysis of the data obtained was carried out using GraphPad prism version 5.0.3. We carried out an analysis of One-Way ANOVA variances for body mass, Two-Ways ANOVA and the Bonferroni post hoc test for relative organ mass and transaminase activity. The number of samples used was n=5 and the significance threshold were set at P<0.05.

3 Results

3.1 Insulin sensitivity test

The glycemic variations in rats in the insulin sensitivity test are shown in Figure 1. These results show that for Group 1 animals with higher fasting blood glucose, the glycemic reduction after 20 minutes after insulin injection is about 15% (330 mg/dl to 279 mg/dl). This reduction is approximately 43% for Group 2 (268 mg/dl to 152 mg/dl), 47% for Group 3 (149 mg/dl to 78 mg/dl), 56% for Group 4 (108 mg/dl to 48 mg/dl) and 61% for NNC (75 mg/dl to 29 mg/dl). In Group 1 rats, even 2 hours after insulin injection, blood glucose remained very high (on average 193 mg/dl) while it decreased by 200% in the NNC group (25 mg/dl). Group 4 animals were therefore less insulin-resistant and were not selected for the experiments.

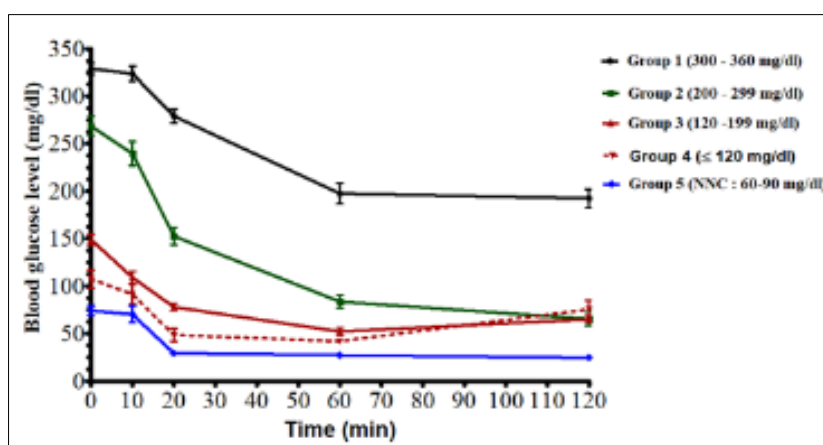


Figure 1 Blood glucose level variation during insulin-sensitivity test of normoglycemic and type 2-induced diabetic rats

The results are expressed as a mean \pm SEM. NNC: normoglycemic negative control.

3.2 Effects of extracts and fractions of *Gymnema sylvestre* on the variation of serum transaminases

These effects are shown in Figure 2. These results show no significant difference ($P > 0.05$) in ALAT activity (UI) between the different groups. On the other hand, they reveal that the level of ASAT (UI) in serum is significantly high ($P < 0.001$)

in the DNC group on day 1 (563.15 ± 8.82) compared to the NNC group (243.60 ± 5.07). Similarly, serum ASAT (UI) levels in the metformin (350.60 ± 23.67), aqueous (381.95 ± 20.41) and methanol (333.41 ± 35.01) and F1 (362.46 ± 24.58), F2 (209.98 ± 9.82) groups were significantly lower ($P < 0.001$) the ASAT rate of the DNC group on day 1 (563.15 ± 8.82). Although the level of ASAT (UI) in the DNC serum on day 14 (468.65 ± 6.20) is lower than that of on day 1, it remains significantly higher ($P < 0.001$) than those in the test lots and the positive metformin control group. In the experimental group treated with the F2 fraction, the decrease is more pronounced so that the ASAT (UI) level of this group (209.98 ± 9.82) is lower ($P > 0.05$) than the NNC group (243.60 ± 5.07).

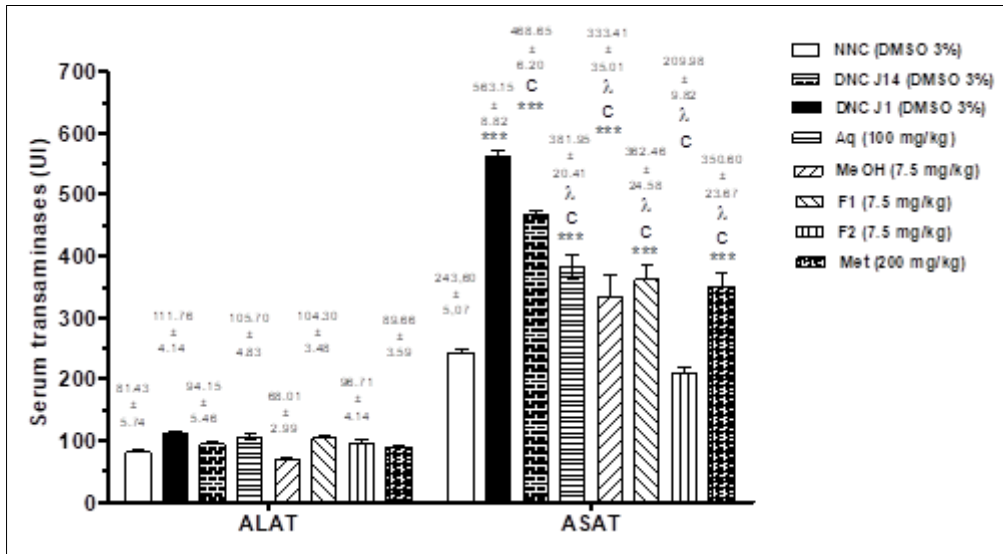


Figure 2 Effects of extracts and fractions of *Gymnema sylvestre* on variation in serum transaminase levels in type 2 diabetes mellitus rats

The results are expressed as a mean \pm SEM (n=5). *** $P < 0.001$: significant difference from the NNC group. °($P < 0.001$): significant difference from the DNC group on Day 1. ^($P < 0.001$): significant difference from the DNC group on Day 1. NNC: normoglycemic negative control; DNC: diabetic negative control; Aq: aqueous extract; MeOH: methanol extract; F1: methylene chloride fraction; F2: methanol fraction; Met: metformin.

3.3 Effects of extracts and fractions of *Gymnema sylvestre* on the variation of renal transaminases.

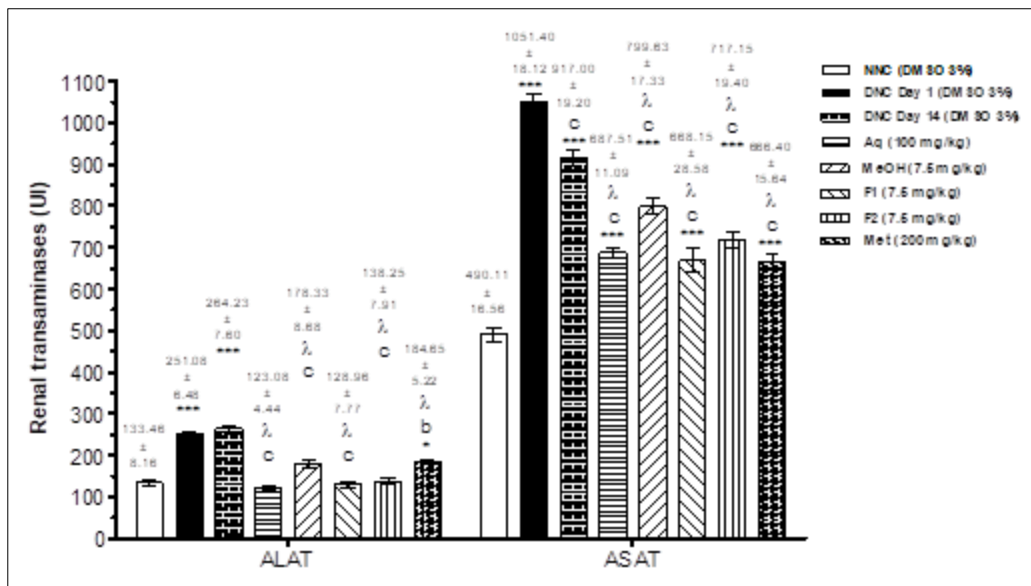


Figure 3 Effects of extracts and fractions of *Gymnema sylvestre* on the variation of renal transaminases in diabetic rats

At the kidney level, ALAT (UI) was significantly elevated ($P<0.001$) in the DNC groups on Day 1 (251.08 ± 6.48) and 14 (264.23 ± 7.60) compared to the NNC group (133.46 ± 8.16) (Figure 3). However, there is no significant difference at the DNC group level between the first and fourteenth days of the experiment. There was also a significant decrease ($P<0.001$) in ALAT (UI) between the DNC group on day 14 and the treated groups metformin (184.65 ± 5.22), aqueous extracts (123.08 ± 4.44), and methanol (178.33 ± 6.68), as well as fractions F1 (128.96 ± 7.77) and F2 (138.25 ± 7.91). However, the decrease is more noticeable among the groups that received Aq, F1 and F2 so that the ALAT rate in these groups is no longer significantly different from that of the NNC group ($P>0.05$).

Figure 3 also shows that renal ASAT (UI) is significantly elevated ($P<0.001$) in the DNC group on day 1 (1051.40 ± 18.12) and 14 (917.00 ± 19.20) compared to the NNC group (490.11 ± 16.56) at the end of treatment. Although the DNC ASAT at day 14 is significantly lower than the DNC ASAT at day 1, it remains significantly higher ($P<0.001$) than in the plant extracts and fractions and NNC groups. However, the ASAT rates of the treated groups are still significantly higher than those of the NNC.

The results are expressed as mean \pm SEM ($n=5$). * ($P<0.05$), *** ($P<0.001$): significant difference from the NNC group. ^b ($P<0.01$) et ^c ($P<0.001$): significant differences from day 1 DNC group. ^λ ($P<0.001$): Significant difference from Day 14 DNC group. NNC: normoglycemic negative control; DNC: diabetic negative control; Aq: aqueous extract; MeOH: methanol extract; F1: methylene chloride fraction; F2: methanol fraction; Met: metformin.

3.4 Effects of extracts and fractions of *Gymnema sylvestre* on the variation of hepatic transaminases

Figure 4 shows a significantly elevated level of hepatic ALAT (UI) ($P<0.001$) in the DNC group on days 1 (304.93 ± 5.66) and 14 (345.70 ± 7.45) compared to the NNC group (188.88 ± 2.32). However, the DNC group on day 14 is significantly high ($P<0.05$) compared to the group on day 1. It is also apparent that the groups treated with Aq extracts (195.88 ± 5.77) and methanol (182.47 ± 16.08), as well as those treated with metformin (178.85 ± 4.98) and F1 fractions (127.51 ± 12.04) and F2 (193.55 ± 5.91) have significantly low ALAT (UI) levels ($P<0.001$) compared to the DNC group on days 1 and 14. These results also show that hepatic ASAT (UI) was significantly elevated ($P<0.001$) in the DNC group on days 1 (738.26 ± 11.09) and 14 (373.33 ± 14.71) compared to the NNC group (154.35 ± 8.34). Although the DNC ASAT rate on Day 14 is significantly low ($P<0.001$) compared to Day 1, it remains significantly higher ($P<0.001$) than the NNC and *G. sylvestre* extracts and fractions. Hepatic ASAT (UI) after treatment with F1 fraction (200.90 ± 14.52) and metformin (203.65 ± 9.84) remained significantly elevated ($P<0.01$) in the NNC group. In contrast, for those treated with aqueous extracts (154.46 ± 8.13), methanol (136.66 ± 7.76) and F2 (160.30 ± 14.44), the difference with the NNC group is no longer significant ($P>0.05$).

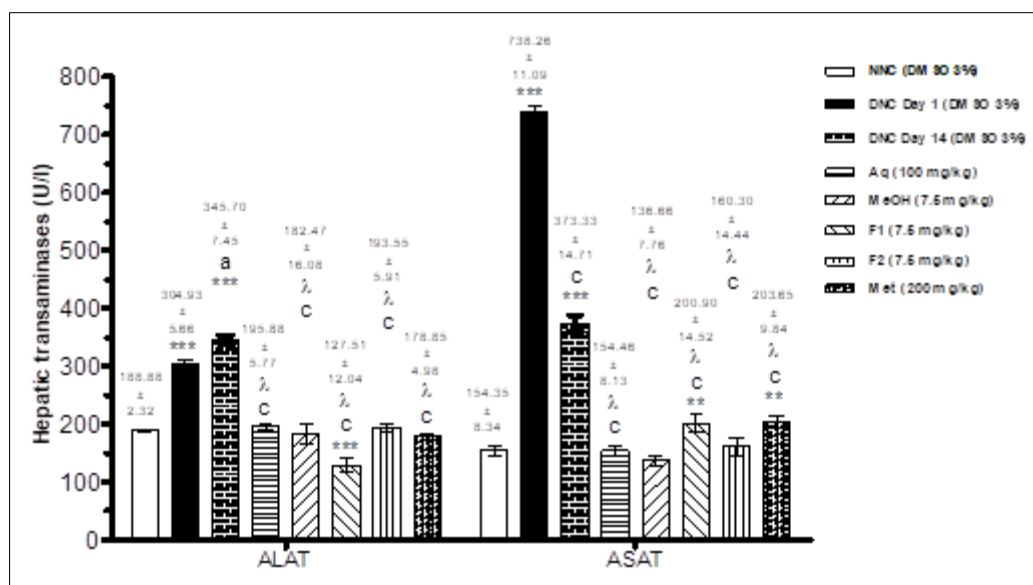


Figure 4 Effects of extracts and fractions of *Gymnema sylvestre* on the variation of hepatic transaminases in diabetic rats

The results are expressed as mean \pm SEM ($n=5$). ** ($P<0.01$) and *** ($P<0.001$): significant differences from the NNC group. ^a ($P<0.05$) and ^c ($P<0.001$): significant difference from the DNC group on day 1. ^λ ($P<0.001$): significant difference from

the DNC group on day 14. DNC: normoglycemic negative control; DNC: diabetic negative control; Aq: aqueous extract; MeOH: methanol extract; F1: methylene chloride fraction; F2: methanol fraction; Met: metformin.

3.5 Effects of extracts and fractions of *Gymnema sylvestre* on body weight variation in type 2 diabetic rats

Figure 5 shows the effect of *G. sylvestre* extracts and fractions on body weight variation in the type 2 diabetic rat. It is apparent from Figure 5A that aqueous and methanol extracts did not induce a significant change in body weight compared to the untreated diabetic group (DNC) although the group of animals treated with methanol extract has a higher weight. In contrast, in the group of animals receiving the oral reference antidiabetic drug (metformin), there was a highly significant reduction ($P < 0.001$) after 14 days of administration compared to the DNC group.

In addition, fractions F1 and F2 showed opposite effects on body weight variation of groups of animals receiving the methylene chloride (significant increase, $P < 0.01$) and methanol fraction (highly significant decrease, $P < 0.001$) respectively, compared to the DNC group on day 14 of treatment (Figure 5B). It should be noted that the F2 fraction had the same effect on body weight variation as the metformin group compared to the untreated diabetes group ($P < 0.001$) after 14 days of treatment.

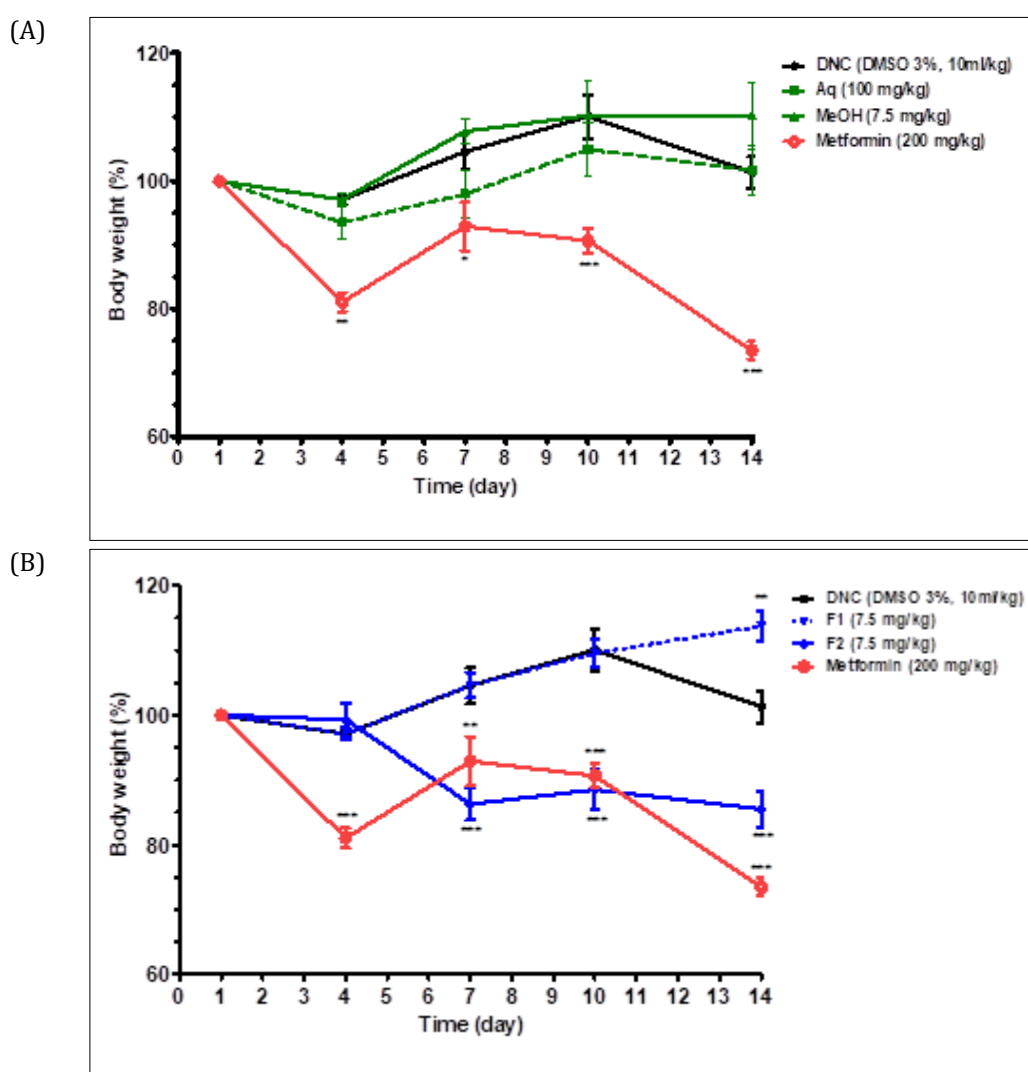


Figure 5 Effects of extracts (A) and fractions (B) of *Gymnema sylvestre* on body weight variation in type 2 diabetic rats.

The results are expressed as mean \pm SEM ($n=5$). *($P < 0.05$); **($P < 0.01$); ***($P < 0.001$): significant difference from the DNC group at the corresponding time. DNC: Diabetic negative control; Aq: aqueous extract; MeOH: methanol extract; F1: methylene chloride fraction; F2: methanol fraction; Met: metformin.

3.6 Effects of extracts and fractions of *Gymnema sylvestre* on changes in relative liver and kidney weights in diabetic rats.

The relative liver weight (g/100 g body weight) of the DNC group on days 1 (4.12 ± 0.05) and 14 (4.23 ± 0.09) increased significantly ($P < 0.001$) compared to the NNC group (2.88 ± 0.09) (Figure 6). After treatment, the relative liver weights of the aqueous (4.13 ± 0.13) and methanol (3.77 ± 0.28) groups and the F1 (4.15 ± 0.05) and metformin (3.93 ± 0.25) fraction remained significantly high ($P < 0.001$) compared to the NNC group.

In addition, after 14 days of treatment, the relative liver weight (g/100 g body weight) of rats treated with the F2 fraction (3.00 ± 0.06) decreased significantly ($P < 0.001$) compared to the DNC group on days 1 and 14, approaching the NNC group value ($P > 0.05$). Regarding the relative kidney weight (g/100 g body weight), no significant differences were observed between groups of animals treated with extracts and fractions of *G. sylvestre* compared to groups NNC and DNC (on days 1 and 14).

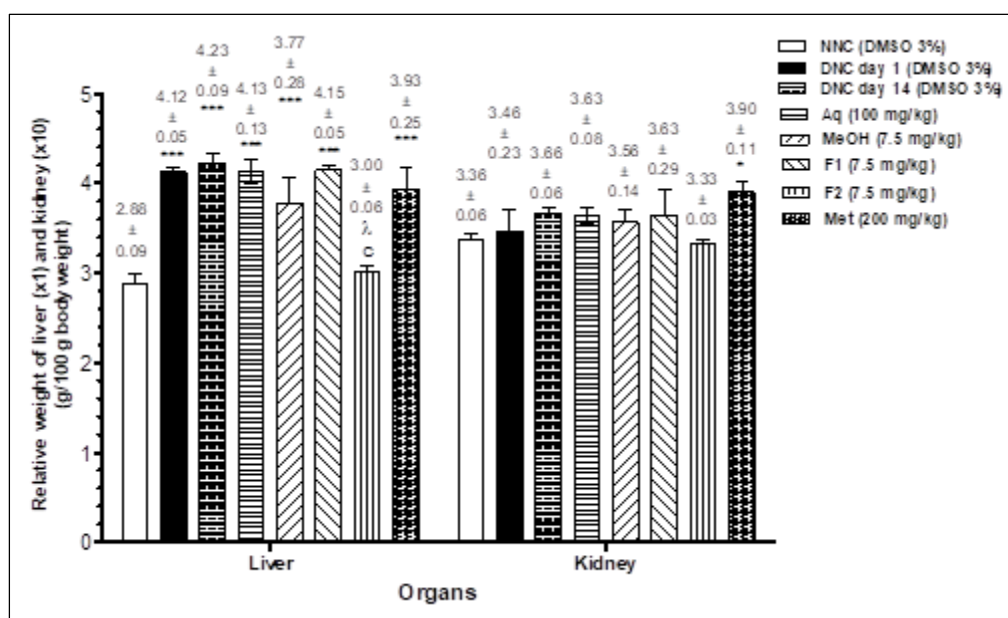


Figure 6 Effects of extracts and fractions of *Gymnema sylvestre* on the relative weight of the liver and kidney (x 10) of the diabetic rat

The results are expressed as mean \pm SEM (n=5). *($P < 0.05$); ***($P < 0.001$): significant difference from the NNC group. $^{\circ}$ ($P < 0.001$): significant difference from DNC group on day 1. $^{\lambda}$ ($P < 0.001$): significant difference from DNC group on day 14. NNC: normoglycemic negative control; DNC: diabetic negative control; Aq: aqueous extract; MeOH: methanol extract; F1: methylene chloride fraction; F2: methanol fraction; Met: metformin.

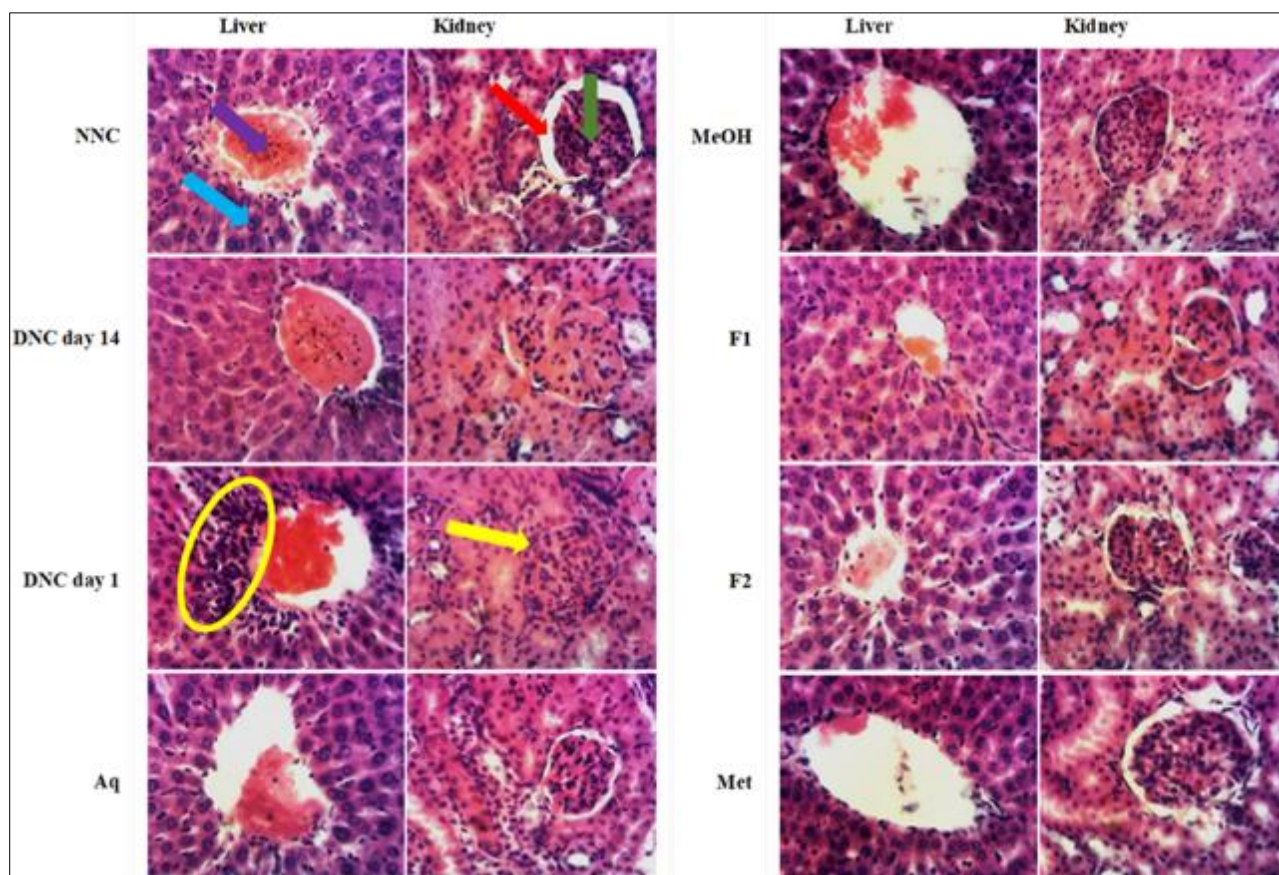
3.7 Effects of extracts and fractions of *Gymnema sylvestre* on histopathology of the liver and kidney of the diabetic rat

Histological sections of the liver (Figure 7) show leukocyte infiltration in the hepatocytes near the centrilobular vein in the DNC group on day 1 and 14. However, this leukocyte infiltration is more pronounced on day 1 compared to day 14. On the other hand, after 14 days of treatment with extracts and fractions of *G. sylvestre* and metformin, there is an almost total regression of the leukocyte infiltration observed in the group of untreated diabetic animals.

At the kidney level (Figure 7), mesangial expansions in urinary space were observed in the DNC groups on Day 1 and 14. This mesangial expansion induced an almost total regression of the urinary space in diabetic animals on the first day of the experiment. In contrast, in groups of diabetic animals treated with the F2 fraction of *G. sylvestre* and Metformin, there is an increase in urinary space close to that of the NNC group.

3.8 Phytochemical analysis of *Gymnema sylvestre*

Phytochemical analysis of extracts and fractions of *G. sylvestre* revealed the presence of tannins, phenols, saponins, flavonoids, alkaloids and the absence of coumarines.



Yellow arrow: Mesangial expansion; Red arrow: Urinary space; Green arrow: Glomerulus; Blue arrow: Hepatocytes; Yellow circle: Leukocyte infiltration; Purple arrow: Centrilobular vein. NNC: normoglycemic negative control; DNC: diabetic negative control; Aq: aqueous extract; MeOH: methanol extract; F1: methylene chloride fraction; F2: methanol fraction; Met: metformin.

Figure 7 Histological appearance of liver and kidney of diabetic rat after 14 days of treatment with extracts and fractions of *Gymnema sylvestre* (Hematoxylin & Eosine x200)

4 Discussion

During these experiments, the activity of extracts and fractions of *G. sylvestre* on the variation of tissue and serum transaminases was evaluated in diabetic insulin-dependent rats induced by a high sucrose diet and dexamethasone injection. The high sucrose diet is associated with a permanent hyperglycemia that is responsible for converting excess glucose into fatty acids, which combine with glycerol to form triglyceride molecules. These are stored in fatty tissue in the form of fat and their deposit on cell membranes is responsible for insulin resistance [1]. Dexamethasone is a drug for therapeutic purposes especially for its anti-inflammatory and immunosuppressive effects [19]. However, it is linked to several side effects including those on glucose metabolism. It is responsible for hepatic neo-glucogenesis, insulin resistance by inhibition of the GLUT 4 transporter (which is the main glucose transporter in the fat tissue and skeletal muscles) and a lack of insulin [19], [20].

In this study, insulin resistance in rats was evaluated on day 1 and it was found that the insulin-resistant animals had fasting blood glucose of 140 mg/dl or greater. This resistance to insulin is therefore due to the combined actions of 12 weeks of high sucrose diet and 5 days of consecutive dexamethasone injection. In addition, the restorative activity of extracts and fractions of *G. sylvestre* was evaluated by measuring the activity of serum, renal and hepatic transaminases in insulin-resistant rats. Diabetes mellitus in general and insulin resistance in particular, is linked to several complications affecting various organs including the kidneys [21]. Diabetic nephropathy is the most serious of the microvascular complications of diabetes and manifests itself in lesions of the renal tubules [7]. One of their characteristics is the increased activity of renal transaminases [6].

In our study, ALAT and ASAT activities at the renal level were very significantly elevated ($P < 0.001$) in diabetic control rats on days 1 and 14 compared to normal rats. These lesions are therefore due to the combined actions of the high sucrose diet and dexamethasone as proved in 2009 by Ramkumar et al which revealed the protective effect of *G.*

montanum, plant of the same genus as *G. sylvestre*, against renal lesions induced by Dexamethasone [14]. The increase in renal activity of transaminases in the diabetic rat was also demonstrated by the studies of Babu and Srinivasan in 1998 which revealed that an increase in the activity of ALAT and ASAT is associated with proximal and distal renal tubule lesions respectively [6]. The present study shows a simultaneous increase in the activity of ALAT and ASAT which could thus testify to a general impairment of the renal tubule. After treatment of diabetic rats with extracts and fractions of *G. sylvestre*, the activity of these transaminases decreased significantly compared to those of the untreated diabetes group on day 1 and day 14. This could therefore be due to the fact that the extracts and fractions counteracted the renal damage caused by the high sucrose diet and dexamethasone.

In addition, insulin resistance causes diffuse or focal accumulation of fat, primarily triglycerides, in the cytoplasm of hepatocytes, resulting in the development of non-alcoholic fatty liver disease [16], [22]. This cytotoxicity is materialized among other things by the modification of the activity of liver enzymes including ALAT and ASAT which is increased [23], [24]. In this study, this trend was observed because hepatic ALAT and ASAT activities were very significantly elevated ($P < 0.001$) in diabetic rats on days 1 and 14 compared to normal rats. In the treated groups, transaminase activity was significantly low ($P < 0.001$) compared to the untreated diabetes group on days 1 and 14, thus testifying to the effectiveness of different treatments in the functional restoration of hepatocytes.

The organic lesions that accompany insulin resistance are also associated with increased transaminase activity in body fluids including blood. Indeed, Babu and Srinivasan showed in their study that kidney lesions were accompanied by an increase in the activity of ALAT and ASAT in the urine [6]. A more recent study by Tolman et al, confirmed the association between liver damage and increased serum activity of ALAT and ASAT [25]. In our study, we found no significant difference in ALAT activity between normoglycemic rats, untreated diabetics and treated diabetics. In contrast, ASAT activity increased significantly ($P < 0.001$) in the untreated group of diabetic animals on days 1 and 14 compared to the normoglycemic group of rats and treated diabetics.

Among the many side effects of dexamethasone, are proteolysis, lipolysis and appetite reduction and therefore food intake [20]. These result in weight loss. This study showed weight loss in virtually all animals during the administration of dexamethasone. This weight reduction continued during the 3 days following cessation of dexamethasone administration.

However, during the remainder of treatment, we found that the weight gain in animals in the aqueous extract, methanol fraction and metformin groups was significantly low ($P < 0.001$) compared to those in the untreated diabetic group. These effects on body mass have also been reported in other studies, including Fatani et al, which showed that administration of *G. sylvestre* ethyl extract was responsible for weight loss in streptozotocin-induced diabetic rats [26]. The aqueous extract and methanol fraction of *G. sylvestre* could thus be used for slimming purposes. In addition, the decrease in relative liver weight in rats treated with F2 could be seen as the result of a reduction in insulin-resistant liver inflammation.

Histopathological investigation in this study revealed hepatic leukocyte infiltration and mesangial expansions in the urinary tract in the untreated group of diabetic animals. These histological abnormalities are almost absent in groups of animals treated with fractions (F1 and F2) of *G. sylvestre* and Metformin. This finding corroborates the different transaminase activities observed in this study, thus testifying to the hepatoprotective and nephroprotective effects of extracts and fractions of *G. sylvestre*. These effects could be the result of the action of the compounds contained in these extracts and fractions and detected in this study by phytochemical screening including saponins and flavonoids. Indeed, saponins, flavonoids and anthraquinones have proven their cytoprotective and anti-inflammatory effects [27], [28]

5 Conclusion

The combination of a high sucrose diet with dexamethasone injection allows the installation of insulin resistance, then type 2 diabetes mellitus in rats. Extracts and fractions of *G. sylvestre* contain tannins, phenols, saponins, flavonoids, and alkaloids. The presence of these secondary metabolites could be responsible for the pharmacological effects observed, in particular the reduction of the level of transaminase in the type 2 diabetic rat at the renal, hepatic and serum level (ASAT) on the one hand, and on the other hand, renal and hepatic (ALAT) levels. The study found that the fractions appear to be more effective than the extracts, as the methanol fraction is the most effective and will need to be tested at multiple doses to determine the effective dose.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Statement of ethical approval

All the animal experiments were carried out in accordance with the animal research: reporting of in vivo experiments (ARRIVE) guidelines and European Union (EU) directive 2010/63/EU for animal experiments and Cameroon National Ethic Committee (ref. FWIRB 00001954).

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