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Antihyperglycemic, antioxidant, and organ protective effects of *Schumanniophyton magnificum* stem bark aqueous extract in dexamethasone-induced insulin resistance rats

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Abstract

This study aimed to evaluate the effect of *Schumanniophyton magnificum* stem bark aqueous extract in dexamethasone-induced insulin-resistant male rats. Firstly, a phytochemical screening of the aqueous extract was carried out. Thereafter, using acute and subacute studies (11 days), the effect of the extract (200 mg/kg and 400 mg/kg) was evaluated on dexamethasone-induced hyperglycemic rats. Glycemia was measured before and after treatment in both studies. Histological examinations for isolated liver, kidneys, and pancreas were performed, body and the weight of some internal organs was determined. The biochemical assay in the blood samples was performed only for the subacute study. Phytochemical analysis revealed that the extract contains phenolic compounds, flavonoids, anthocyanins, saponins, gallic tannins, coumarins, and anthraquinones. In both studies, *Schumanniophyton magnificum* stem bark aqueous extract reduced the glucose blood Area under the Curve produced by dexamethasone injection. The extract, as well as glibenclamide significantly lowered the dexamethasone-induced increase in transaminases activities and uric acid concentration. Superoxide dismutase activity increased in all extract and glibenclamide groups compared to the dexamethasone group. The extract effect on the glutathione concentration was dose-dependent ($p < 0.05$ and $p < 0.001$ respectively). The histology of organs from rats treated with dexamethasone revealed hepatic cytolysis, leukocyte infiltration, and islet hypotrophy. The extract and glibenclamide-treated groups had fewer or no anomalies observed with dexamethasone administration. Aqueous extract of *S. magnificum* stem bark protects against dexamethasone-induced pancreatic and hepatorenal abnormalities, probably due to the antioxidant properties of the chemical groups present in this extract.

Keywords: *Schumanniophyton magnificum* extract; Stem bark; Antioxidant; Insulin resistance; Dexamethasone

1. Introduction

Schumanniophyton magnificum, also known as "Tsit Modo" in Cameroon's Ewondo language or "akito" in Nigeria's Igbo's language is a little tree that grows in the tropical zones of West and Central Africa. It is mostly found in Cameroon, Sierra Leone, southeastern Nigeria (Calabar and Igbogodo), and Ghana's lowland rainforest areas [1].

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Schumanniophyton magnificum bark decoction is utilized in an enema to treat dysentery in Cameroon [2]. It is often used as a post-circumcision lotion [3]. The stem bark has been used for a long time by forest workers for the treatment of snake bites and stomach upset [1, 4]. The juice of stem bark of *Schumanniophyton magnificum* is applied to the bite to treat scorpion sting [5]. Fever, malaria, dysmenorrhoea, and some cases of female infertility are all treated using the stem bark of *Schumanniophyton magnificum* in combination with the stem bark of *Albizia zygia* [6]. *Schumanniophyton magnificum* stem bark aqueous extract promotes puberty and gonadotropin production, as well as ovarian folliculogenesis and fertility in immature rats [7]. The stem bark is also used in Congo to cure blennorrhoea, syphilitic cankers, and ulcers as an antiseptic. The roots are employed in the treatment of insanity [8]. The bark infusion is used to treat stomach aches, as a purgative or vermifuge [9]. This plant is also well-known for its snake-repelling properties [8].

Petroleum, chloroform, ethanol (absolute), and methanol *Schumanniophyton magnificum* stem bark extracts exhibited *in vivo* inhibitors activity against phospholipase A2 [10, 11]. A peptide produced from *Schumanniophyton magnificum* stem bark aqueous extract, which was comparable in amino acid composition to the cardiotoxins included in snake venom, reduced the effects of cardiotoxin and total venom of cobra species in a dose-dependent manner [12]. Antiviral efficacy against HIV and HSV revealed that schumannificine 1 (the chromone secondary amine isolated from a methanolic extract of the root bark) had the best antiviral activity against HIV, while a variety of its derivatives had powerful anti-HSV activity [1]. *S. magnificum extracts* are active against *Plasmodium falciparum* [6].

Several phytochemical studies on this species have yielded the isolation of a variety of chromone alkaloids, including schummaniophytine, isoschummaniophytine, N-methyl schummaniophytine, schumaginine, and schumannificine, as well as the related bases trigonelline, rohitukine, and the chromone noreugenin [1]. New chromone glycosides and schummaniofioside A and B have been discovered in the n-butanol extract of the cameroonian species' root bark [2]. Noreugenin and ß-sitosterol were extracted from a methanolic extract of *Schumanniophyton magnificum* stem bark, and noreugenin was found to have antibacterial activity against *S. typhimurium*, with an inhibition diameter of 11 mm [13].

The current study looked at the antihyperglycemic, antioxidant, and protective effects of *Schumanniophyton magnificum* stem bark aqueous extract on the pancreas, liver, and kidneys in dexamethasone-induced insulin resistance in rats.

2. Material and methods

2.1. Plant material collection and Extraction

The National Herbarium of Cameroon authenticated fresh *Schumanniophyton magnificum* stem bark obtained from a phytotherapist at the Mvog-Atangana Mballa Market in Yaoundé (Cameroon) and compared it to specimen No. 6511/HNC of Cameroon National Herbarium. They were cleaned, dried, and crushed into a fine powder in the Laboratory of Endocrinology and Radioisotopes of the Institute of Medical Research and Medicinal Plants Studies.

300 g of *Schumanniophyton magnificum* stem bark powder was weighed and brought to a boil in 3.5l of distilled water for 15 minutes and cooled to room temperature. The solution thus obtained was first left to stand for decantation, then the supernatant was filtered first with cotton, to remove coarse particles, and finally with Whatman No. 4 paper. The filtrate thus obtained was evaporated in an oven at 60°C, until completely dried. *S. magnificum stem* bark aqueous extract was obtained from the dried product. The extracted weight was 60 g, indicating a yield of 20% w/w.

2.2. Phytochemical screening

Harbone's methods were used to carry out the phytochemical screening [14].

2.3. Experimental animals

Wistar male albino rats weighing 170 g to 260 g were used in the experiment. These rats were obtained from the University of Yaoundé I's Higher Teachers' Training College (ENS) animal house (Cameroon). They were fed a conventional rat diet and free access to water. They were kept in a controlled environment with a 12 h light/12 h dark cycle and a controlled ambient temperature of 25 °C ± 2 °C, 50 % ± 10 % relative humidity. The animal study was conducted during January 2020, at the Endocrinology and Radio-Isotopes Laboratory of the Institute of Medical Research and Medicinal Plants Studies (IMPM), Yaoundé, Cameroon. The research was carried out with the agreement of the Institutional Animal Ethics Committee and following the guidelines of the Cameroon National Ethical Committee (Reg. No. FWA-IRB00001954).

2.4. Drugs and Chemicals

Glibenclamide was provided by the Mahalakshmi chemicals in Hyderabad, Telangana, India. Diethyl ether (Ether Gifrer Solution) was purchased with a labeled amount of FL/400ML from a local pharmacy. The other chemicals used were of analytical grade and were used exactly as they were given to us.

2.5. Acute effects of *Schumanniophyton magnificum* stem bark aqueous extract on dexamethasone-induced insulin resistance

12 h fasted rats were randomized into five groups of six each (n = 6). Vehicle (10ml/kg) and normal saline (1ml/kg, i.p.) were given to rats of Group I (Control). Group II (DEXA) rats received vehicle (10ml/kg) gavage and dexamethasone (8 mg/kg; i.p.), Group III (GLIB) rats received glibenclamide (5 mg/kg) and dexamethasone (8 mg/kg; i.p.), Group IV (SM200) rats received gavage of 200 mg/kg of *Schumanniophyton magnificum* extract and dexamethasone (8 mg/kg; i.p.), Group V (SM400) was given dexamethasone (8 mg/kg; i.p.) and gavage of 400 mg/kg of *Schumanniophyton magnificum* extract. 30 minutes after oral gavage of vehicle, glibenclamide, or extract, normal saline or dexamethasone was given. Animals were then submitted to an Oral Glucose Tolerance Test (OGTT) after being given dexamethasone 4 hours after. Blood glucose levels were measured 30, 60, and 120 minutes after glucose (2 g/kg) was administered.

2.6. Subacute study of the protective effects of *Schumanniophyton magnificum* stem bark aqueous extract on dexamethasone-induced insulin resistance

2.6.1. Experimental procedure

For 11 days, rats were randomly divided into five groups of six animals (n = 6) each and given different treatments once a day. In all this experiment, dexamethasone was administered intraperitoneally at 8 mg/kg. Group I (Control) rats received vehicle (10ml/kg) and normal saline (1ml/kg; i.p.), Group II (DEXA) rats received vehicle 10 ml/kg and dexamethasone, Group III (GLIB) rats received orally glibenclamide (5mg/kg) and dexamethasone, and Group IV (SM200) rats received *Schumanniophyton magnificum* extract at 200mg/kg and dexamethasone, while Group V (SM400) rats received *Schumanniophyton magnificum* extract at 400mg/kg and dexamethasone. During the study period, dexamethasone was given to Group II, Group III, Group IV, and Group V rats on the day 7^{th} to the day 11^{th}. Dexamethasone was given 30 minutes after the vehicle or extract was gavaged [15]. The body weight of the animals was recorded daily during the study.

Animals fasted for 12 hours at the end of the experiment. Under light ether anesthesia, blood samples were taken through retro-orbital plexus puncture. The blood samples were centrifuged for 25 minutes at 2500 RPM. Biochemical analysis was carried out on the isolated serum. The Oral Glucose Tolerance Test (OGTT) was then performed in rats. The rats were subsequently anesthetized with ether and sacrificed after the OGTT. Each rat's pancreas, adrenal glands, kidney, and liver were removed and weighed. Liver, kidney, and pancreas tissues were dissected and preserved in formalin (10 %), for subsequent histological studies.

2.6.2. Estimation of biochemical parameters

Commercial kits provided by Biolabo S.A.S., Les Hautes Rives, 02160 Maizy, France, were used to measure serum transaminases ((alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT)) activities, as well as uric acid concentration. The activity of superoxide dismutase (SOD) was determined using the Misra and Fridovish method [16]. Ellman's procedure was used to determine reduced GSH [17].

2.6.3. Oral Glucose Tolerance Test (OGTT)

The animals were starved for 12 hours following the final treatment dosage on the eleventh day, after which the first blood glucose level was determined using a CERA-CHEK brand glucometer on blood obtained from the retro-orbital plexus via a capillary tube. After then, the rats were given glucose (2g/kg). The blood glucose levels were then measured 30, 60, and 120 minutes after the glucose solution was administered.

2.7. Histopathology examination

Following the macroscopic investigation, representative fragments of the liver, the left kidney, and the pancreas were fixed in a 10 % solution of buffered formalin (pH 7.4) and encased in paraffin, using Mayer's technique [18], with minor changes, and examined under a light microscope. For the investigation of tissue alterations under an optical microscope (100 X or 200 X), five-micrometer slices were obtained and colored with Hematoxylin-Eosin (HE).

2.8. Statistical analysis

The data were presented as a mean ± standard error of the mean (SEM). GraphPad Prism software version 5.03 was used to analyze the data. The One-Way ANOVA test was used to compare the data, followed by the Dunnett test. At $p<0.05$, the difference was considered statistically significant.

3. Results

3.1. Phytochemical screening

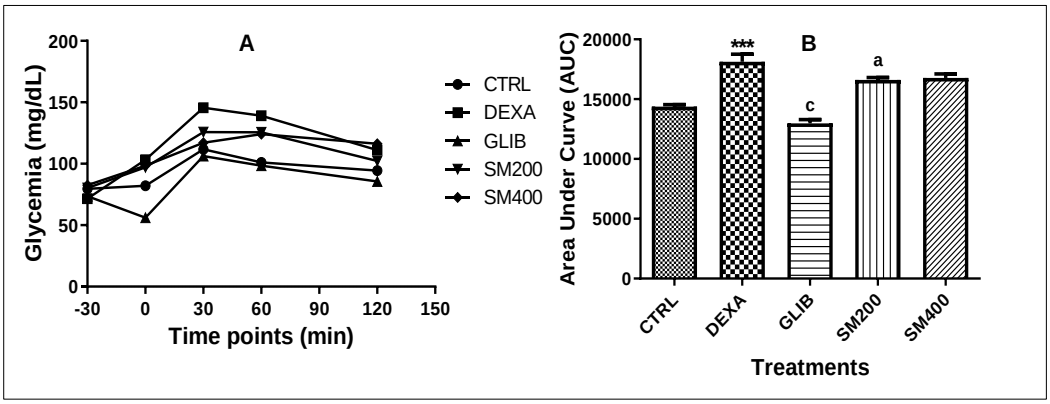
Chemical analysis showed that the aqueous extract of *Schumanniophyton magnificum* stem bark contains phenolic compounds, flavonoids, anthocyanins, saponins, gallic tannins, coumarins, and anthraquinones (Table 1).

Table 1 Phytochemical screening results of aqueous extract of *Schumanniophyton magnificum* stem bark

Phytochemicals classes	Result of the test
Alkaloids	-
Phenolic compounds	+
Triterpenes	-
Steroids	-
Flavonoids	+
Anthocyanins	+
Glucosides	-
Saponins	+
Gallic tannins	++
Coumarins	+
Anthraquinones	+

Legend: -: Absent; +: present; ++: moderate presence.

3.2. *Schumanniophyton magnificum* stem bark aqueous extract exerts acute hypoglycemic effects on dexamethasone-induced hyperglycemia



CTRL: rats given distilled water and 0.9% NaCl; **DEXA:** rats given distilled water and dexamethasone (8 mg/kg); **GLIB:** rats given glibenclamide (5 mg/kg) and dexamethasone; **SM200:** rats treated with the extract at 200 mg/kg and dexamethasone; **SM400:** rats treated with the extract at 400 mg/kg and dexamethasone. Curve points and bars of AUC represent the mean±ESM, n = 6. ***p <0.001 determined with Dunnett's post-test compared to rats in the CTRL group, $^{a}p<0.05$; $^{c}p<0.001$ significant difference compared to rats in the Dexamethasone group, following one-way ANOVA.

Figure 1 Oral Glucose Tolerance Test (OGTT) in Acute Study (A) and the associated Area under the Curve (AUC) (B)

Figure 1A depicts the mean baseline glucose levels and blood glucose fluctuation curve in the acute study's oral hyperglycemia test. The results demonstrated that basal blood glucose levels ranged from 61 to 92mg/dL. When compared to the control group (CTRL), the Area Under the Curve associated with the Oral Glucose Tolerance Test

(OGTT) showed an important rise in blood glucose in rats treated with dexamethasone and distilled water (14380±156.9 to 18120±631.1 (p<0.001).

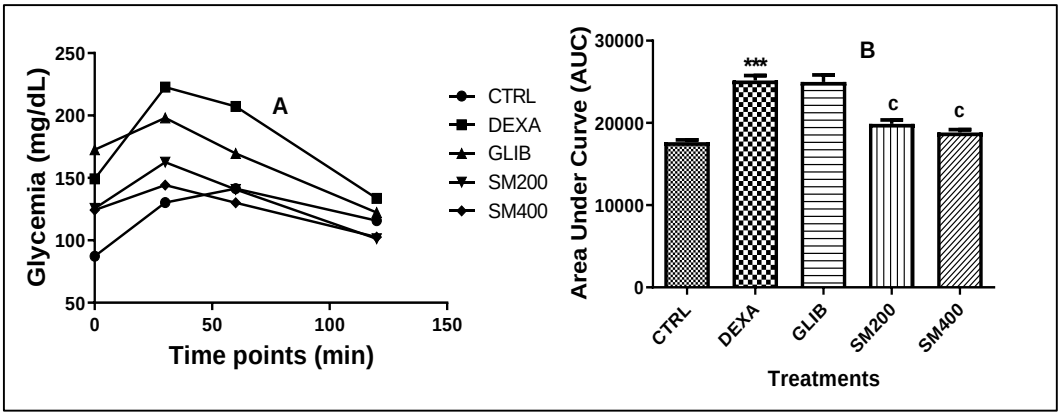
As compared to rats treated with dexamethasone only, rats pretreated with glibenclamide or the extract (200 mg/kg) respectively showed a significant decrease in AUC (18120±631.1 to 12970±313.8; p<0.001) for glibenclamide and 18120±631.1 to 16601±211.3 (p<0.05) for extract (200 mg/kg) (Figure 1B).

3.3. Subacute study of the protective effects of *Schumanniophyton magnificum* stem bark aqueous extract on dexamethasone-induced insulin resistance

3.3.1. Effects of Schumanniophyton magnificum stem bark aqueous extract on the blood glucose levels of insulin-resistant rats

Baseline glycemia ranged from 80 to 170mg/dL and blood glucose fluctuation curves in the Oral Glucose Tolerance Test performed at the end of the subacute study are shown in Figure 2A.

The Area under the Curve linked with the OGTT demonstrated a significant increase in the rats treated with dexamethasone when compared to the control group (17667±276 to 25151±605, p<0.001). When compared to the group of rats treated with only dexamethasone (DEXA), the rats pretreated with the plant extract showed a significant drop in AUC of blood glucose levels curve (25151±605 to 17667±276 and 25151±605 to 18844±332.5, respectively for 200 mg/kg (p<0.001) and 400 mg/kg (p<0.001) (Figure 2B).



CTRL: rats given distilled water and 0.9% NaCl; **DEXA:** rats given distilled water and dexamethasone (8 mg/kg); **GLIB:** rats given glibenclamide (5 mg/kg) and dexamethasone; **SM200:** rats treated with the extract at 200 mg/kg and dexamethasone; **SM400:** rats treated with the extract at 400 mg/kg and dexamethasone. Curve points and bars of AUC represent the mean±ESM, n = 6. ***p <0.001 determined with Dunnett's post-test compared to rats in the CTRL group, $^{c}p < 0.001$ significant difference compared to rats in the Dexamethasone group, following one-way ANOVA.

Figure 2 Oral Glucose Tolerance Test (OGTT) in the Subacute Study (A) and the associated Area Under the Curve (AUC) (B)

3.3.2. Effect of Schumanniophyton magnificum stem bark aqueous extract on body weight

Table 2 displays that all groups showed the same rise in body variation from day 1 to day 7. On day 8, the dexamethasone-treated rats showed a drop in body weight change (8.76±2.52 to 1.34±0.97; p<0.05) as compared to the control group. This decline continues until the day 12 (2.88±2.38 to -13.37±1.14, p <0.001).

Table 2 Change in body weight in the subacute study (% variation)

Days	Group				
	CRTL	**DEXA**	**GLIB**	**SM200**	**SM400**
Day 1	0.00	0.00	0.00	0.00	0.00
Day 3	6.10±0.88	7.01±0.34	5.53±0.86	6.17±0.62	5.30±1.19
Day 5	7.52±1.33	7.94±0.77	7.69±1.66	6.46±0.81	6.04±0.96

Day 7	9.41±2.07	8.55±0.82	8.64±2.51	7.64±0.97	6.67±1.21
Day 8	8.76±2.52	1.34±0.97*	4.22±2.11	2.76±0.97	1.13±1.20
Day 9	7.12±2.98	-2.33±0.63**	-0.93±1.64	-2.53±1.20	-4.23±1.46
Day 10	8.26±2.49	-5.84±0.80***	-6.75±1.58	-5.87±0.80	-6.77±1.48
Day 11	7.33±2.81	-9.08±1.02***	-9.02±1.28	-9.04±0.85	-9.24±1.36
Day 12	2.88±2.38	-13.37±1.14***	-14.02±1.27	-13.42±0.84	-13.04±1.46

CTRL: rats given distilled water and 0.9% NaCl; **DEXA:** rats given distilled water and dexamethasone (8 mg/kg); **GLIB:** rats treated with glibenclamide (5 mg/kg) and dexamethasone; **SM200:** rats treated with the extract at 200 mg/kg and dexamethasone; **SM400**: rats treated with the extract at 400 mg/kg and dexamethasone. Each value represents the percentage change mean of body weight±ESM, n = 6. *p <0.05; **p <0.01; ***p <0.001 determined with Dunnett's post-test compared to rats in the CTRL group, following one-way ANOVA.

3.3.3. Effect of Schumanniophyton magnificum stem bark aqueous extract on the weight of organs

The liver, pancreas, kidneys, and adrenals are organs whose relative weight has been determined. The relative liver weights of rats treated with dexamethasone only increased significantly (2.76±0.12 to 3.968±0.30; p <0.001), when compared to the control group. When rats in the dexamethasone group were compared to those in the control group, the relative kidney weight increased (0.64±0.03 to 0.77±0.04, p<0.05). Dexamethasone subacute injection reduced the relative weight of the adrenals (0.02±0.00 to 0.01±0.00, p<0.05) (Table 3).

Table 3 Effect of *Schumanniophyton magnificum* stem bark aqueous extract on relative organ weight at the end of the study (% b.w)

Organs	**Group**				
	CTRL	**DEXA**	**GLIB**	**SM200**	**SM400**
Liver	2.76±0.12	3.97±0.30**	4.04±0.13	3.88±0.25	3.64±0.22
Kidneys	0.64±0.03	0.77±0.04*	0.82±0.04	0.72±0.03	0.70±0.03
Pancreas	0.42±0.05	0.35±0.03	0.33±0.05	0.314±0.04	0.31±0.04
Adrenals	0.018±0.00	0.013±0.00*	0.014±0.00	0.014±0.00	0.013±0.00

CTRL: rats given distilled water and 0.9% NaCl; **DEXA:** rats given distilled water and dexamethasone (8 mg/kg); **GLIB:** rats treated with glibenclamide (5 mg/kg) and dexamethasone; **SM200:** rats treated with the extract at 200 mg/kg and dexamethasone; **SM400:** rats treated with the extract at 400 mg/kg and dexamethasone. The data in the table represent the relative mean values of organ weights±ESM; n = 6. *p <0.05; **p <0.01 determined with Dunnett's post-test compared to the CTRL group, following one-way ANOVA.

3.3.4. Schumanniophyton magnificum stem bark aqueous extract improves liver and renal function markers and antioxidant status of insulin-resistant rats

When compared to the control group (CTRL), the activity of Aspartate aminotransferase (ASAT) in rats treated with dexamethasone increased significantly (59.03±3.67 to 217.2±8.89; p<0.001). On the contrary, a significant decrease (p<0.001) in ASAT activity was detected in rats pretreated with glibenclamide (217.2±8.89 to 202.81±11.00) and those treated with plant extract (217.2±8.89 to 11.02±0.38 (p<0.001) for 200 mg/kg and 217.2±8.89 to 20.02±1.08 (p<0.001) for 400 mg/kg respectively (Table 4).

When compared to the control group, rats treated with dexamethasone showed a significant increase in alanine aminotransferase (ALAT) activity (p<0.001). At a dose of 200 mg/kg, the plant extract significantly reduced ALAT activity elevated by dexamethasone (146.5±6.41 to 121.22±5.68; p<0.05) (Table 4).

A significant increase in uric acid concentration was observed in the dexamethasone group (12.07±0.73 to 18.50±0.57, p<0.001) versus the control group. The plant extract at 200 mg/kg significantly lowered uric acid level compared to the group given dexamethasone (DEXA) (18.50±0.57 to 11.02±0.38; p <0.001) (Table 4).

Rats given dexamethasone had significantly lower superoxide dismutase (SOD) activity (p<0.001) than those of the control group (0.19±0.00 to 0.15±0.00, p<0.001). In comparison to SOD activity in the dexamethasone-treated group, there was a significant increase induced by the administration of glibenclamide (0.15±0.00 to 0.18±0.00; p<0.001) or *S. magnificum* extract at 200 mg/kg (0.15±0.00 to 0.17±0.00; p<0.001) and 400 mg/kg (0.15±0.00 to 0.16±0.00; p<0.01) (Table 4).

In comparison with those of the dexamethasone group, rats given *S. magnificum* extract exhibited an increase in GSH levels from 1.67±0.18 to 2.53±0.24 ($p<0.05$) and 1.67±0.18 to 3.35±0.32 ($p<0.001$) respectively at 200 mg/kg and 400 mg/kg (Table 4).

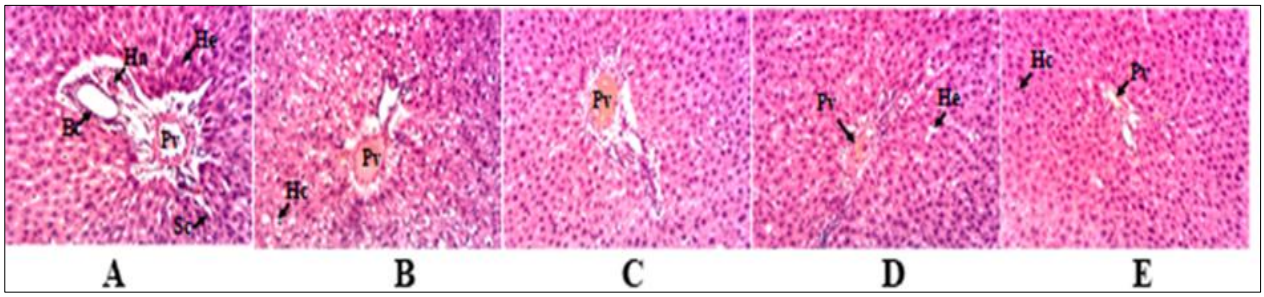
Table 4 Effect of *S. magnificum* stem bark aqueous extract on biochemical parameters of dexamethasone-induced insulin-resistance rats

Biochemical parameters	Group				
	CTRL	DEXA	GLIB	SM200	SM400
ASAT (UI/L)	59.03±3.67	217.2±8.89***	202.8±11.00^{c}	11.02±0.38^{c}	20.02±1.08^{c}
ALAT (UI/L)	66.84±3.23	146.5±6.41***	144.6±8.58	121.2±5.68^{a}	140.2±9.31
Uric acid (mg/L)	12.07±0.73	18.50±0.57***	20.64±0.097	11.02±0.38^{c}	20.02±1.08
SOD (U/mg protein)	0.19±0.00	0.15±0.00***	0.18±0.00^{c}	0.17±0.00^{c}	0.16±0.00^{b}
GSH (Mmol/mL)	1.85±0.04	1.67±0.18	1.96±0.21	2.53±0.24^{a}	3.35±0.32^{c}

CTRL: rats given distilled water and 0.9% NaCl; **DEXA:** rats given distilled water and dexamethasone (8 mg/kg); **GLIB:** rats given glibenclamide (5 mg/kg) and dexamethasone; **SM200:** rats treated with the extract at 200 mg/kg and dexamethasone; **SM400:** rats treated with the extract at 400 mg/kg and dexamethasone. All values are expressed as mean±SEM, n = 6. ***p <0.001 determined with Dunnett's post-test compared to rats in the CTRL group, $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ significant difference compared to rats in the Dexamethasone group, following one-way ANOVA.

3.3.5. Effects of the aqueous extract of Schumanniophyton magnificum stem bark aqueous extract on the histology of the pancreas, liver, and kidneys of rats at the end of the subacute study.

For rats belonging to the control group (CTRL), or those pretreated with glibenclamide (GLIB) or plant extract at 200 mg/kg (SM200), histological examination of the liver revealed a normal appearance of liver tissue. Dexamethasone-treated rats (DEXA) showed hepatocyte cytolysis, as also shown by those pretreated with plant extract at 400 mg/kg (SM400), even though pronounced in the latter (Figure 3).

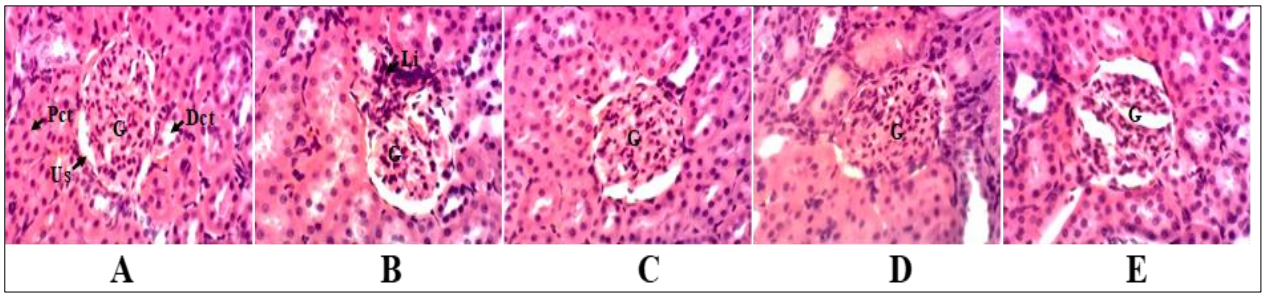


A = CTRL; **B** = DEXA; **C** = GLIB; **D** = SM200; **E** = SM400; **Pv** = portal vein; **He** = Hepatocyte; **Sc** = Sinusoidal capillary; **Ha**= hepatic artery; **Bc** = Biliary canal; **Hc** = Hepatocyte cytolysis; **Lv**: lipid vacuoles. **CTRL:** rats given distilled water and 0.9% NaCl; **DEXA:** rats given distilled water and dexamethasone (8 mg/kg); **GLIB:** rats treated with glibenclamide (5 mg/kg) and dexamethasone; **SM200**: rats treated with plant extract at 200 mg/kg and dexamethasone; **SM400**: rats treated with plant extract at 400 mg/kg and dexamethasone

Figure 3 Photomicrographs of the Liver (H&E. 100X)

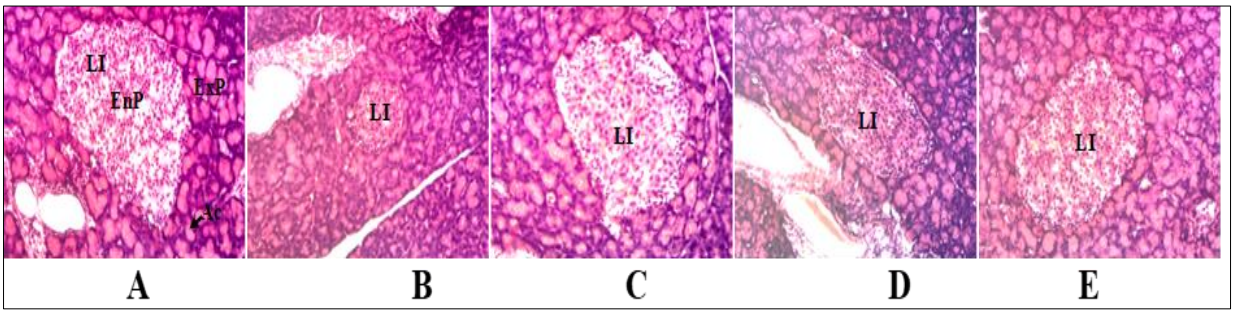
In rats treated with dexamethasone (DEXA), histological examination of the kidneys revealed leukocyte infiltration and a reduction in glomerular cell density. Glibenclamide or extract-treated rats had normal renal parenchyma (Figure 4).

The pancreas histological study highlighted a hypotrophy of the islets pancreas in rats treated with dexamethasone; the other groups did not show any alteration of pancreatic tissue (Figure 5).



A = CTRL; **B** = DEXA; **C** = GLIB; **D** = SM200; **E** = SM400; Kidney; **G** = Glomerulus; **Us** = urinary space; **Dct** = distal collecting tubule; **Pct** = proximal collecting tubule; **Li** = Leukocyte infiltration. **CTRL:** rats given distilled water and 0.9% NaCl; **DEXA:** rats given distilled water and dexamethasone (8 mg/kg); **GLIB:** rats treated with glibenclamide (5 mg/kg) and dexamethasone; **SM200**: rats treated with plant extract at 200 mg/kg and dexamethasone; **SM400**: rats treated with plant extract at 400 mg/kg and dexamethasone

Figure 4 Photomicrographs of the kidney (H&E. 200X)



A = CTRL; **B** = DEXA; **C** = GLIB; **D** = **SM200**; **E** = **SM400**; Pancreas; **LI** = Langerhans Islets; **Ac** = Acini Cell; **EnP** = endocrine pancreas; **ExP** = exocrine pancreas. **CTRL:** rats given distilled water and 0.9% NaCl; **DEXA:** rats given distilled water and dexamethasone (8 mg/kg); **GLIB:** rats treated with glibenclamide (5 mg/kg) and dexamethasone; **SM200:** rats treated with plant extract at 200 mg/kg and dexamethasone; **SM400:** rats treated with plant extract at 400 mg/kg and dexamethasone.

Figure 5 Photomicrographs of the pancreas (H&E. 100X)

4. Discussion

The purpose of this study was to see how dexamethasone-induced insulin resistance in *Wistar* albino rats was affected in acute and subacute treatments by *Schumanniophyton magnificum* stem bark aqueous extract.

The effect on insulin resistance was assessed acutely with an Oral Glucose Tolerance Test (OGTT) at the end of the subacute study. The effect on hepatic and renal functions was measured in a subacute study through the evaluation of some anthropometric parameters and biochemical markers. Oxidative stress indicators were also evaluated and additionally, hepatorenal and pancreatic histology was performed. The OGTT demonstrated a significant increase in blood glucose Area Under the Curve (AUC) in rats treated with dexamethasone when compared to those in the control group, after acute administration. These results are supported with findings of Mawout *et al.* [19], on acute and subacute effects of *Picralima nitida* seeds aqueous extract on dexamethasone-induced insulin resistance in rats, indicating a hyperglycemic effect of dexamethasone. A significant reduction in the AUC of glycemia in rats pretreated with glibenclamide (5 mg/kg) or with 200 mg/kg of the plant extract was seen, suggesting that the *S. magnificum* stem bark aqueous extract may have an anti-hyperglycemic protective effect. The OGTT, which was achieved at the end of the subacute study, revealed no significant difference in AUC between rats treated with glibenclamide and rats treated with dexamethasone. This could be explained by the fact that glibenclamide has an insulin-secreting effect [20]. The insulin resistance induced by dexamethasone is not considered linked to the defect in insulin secretion, but rather to its use by target cells via the decrease in translocation of GLUT 1 and GLUT 4 transporters on the surface of cell membranes [21]. In contrast, a significant, dose-dependent AUC drop in the OGTT curve was observed in rats treated with the *S. magnificum* extract in the subacute study. This finding implies that *S. magnificum's* stem bark extract may improve insulin sensitivity in target cells. Al-Ishaq *et al*. [22] have previously reported the cellular processes and ameliorative effects of flavonoids on blood glucose levels (one of the phytochemicals found in the extract of *S. magnificum)*.

At the end of the subacute phase, the serum total protein concentration (data not shown) revealed no significant difference between the dexamethasone-treated rats and those of the control group. Dexamethasone is well-known for causing weight loss [15]. When compared to the control group, the dexamethasone-treated group experienced loss in body weight from day 8 of the study, until the end of the study, similar to that of other groups treated either with glibenclamide or plant extract. This decrease could indicate a proteolytic effect of dexamethasone. These findings suggest that the plant extract has no protective impact on protein catabolism induced by dexamethasone.

Transaminases, enzymes present inside cells, particularly in the liver and muscles cells, can be used to measure liver function. In rats treated with dexamethasone alone Alanine Aminotransferase (ALT) activity was elevated, as well as relative liver weight, this indicated a change in hepatic function and presence of hypertrophy, due to the toxic effects of dexamethasone. These results are similar to those of Wego *et al*. [23], who investigated the preventive impact of *Baillonella toxisperma* bark aqueous extract on dexamethasone-induced insulin-resistant rats. In contrast to the control group, the activity of Aspartate Aminotransferase (ASAT) was considerably higher in rats treated with dexamethasone. This result agrees with Kumar *et al.*'s [21] investigation, in which dexamethasone dosing also resulted in decreased liver function. The rats pretreated with glibenclamide and those treated with plant extract had significantly lower ASAT activity. These findings are comparable to those of Abou-Seif *et al*. [24], which demonstrated the ameliorative impact of *Thymus vulgaris* extract on dexamethasone-induced liver injury and dysfunction, and would indicate a hepatomodulatory action of *S. magnificum aqueous* extract.

Uric acid level provides information on the degree of kidney filtration and therefore is an indicator of kidney function. It is the ultimate product of purine catabolism. The results of this study demonstrated that rats treated with dexamethasone only have significantly higher uric acid concentrations. The greater uric acid content in the blood might be due to an increase in uric acid production in the body or a decrease in uric acid clearance by the kidneys. Furthermore, relative kidney weights were significantly higher in the same group of rats given dexamethasone alone. These results are consistent with those of Hasona *et al.* [25], who investigated the effect of *Vitis vinifera* aqueous extract on dexamethasone-induced renal and hepatic dysfunctions, finding a decrease in the renal filtration and renal hyperactivity, a marker of oxidative stress in kidney cells, due to dexamethasone. In comparison to rats treated with dexamethasone, rats treated with the extract showed a significant reduction in uricemia at 200 mg/kg. This observation could indicate that the aqueous extract of *S. magnificum* at 200 mg/kg has a protective impact on renal function.

In comparison to the control group, dexamethasone-only-treated rats had considerably lower superoxide dismutase (SOD) activity. SOD is an oxidoreductase that catalyzes the disproportionation of superoxide anions to hydrogen peroxide, resulting in free radical elimination. These findings support the data of Keeney *et al.* [26], who investigated the responses of antioxidant enzymes to hyperoxia in preterm and term rats, following prenatal dexamethasone treatment, demonstrating that dexamethasone causes oxidative stress. The considerable increase in SOD activity in the rats pretreated with the extract suggests that the aqueous extract of *S. magnificum* protects against dexamethasone-induced oxidative stress.

In rats pretreated with the *S. magnificum* extract, there was a considerable increase in the concentration of reduced glutathione (GSH). By removing free radicals and preventing lipid peroxidation, GSH enhances oxidative stress resistance [27]. Given that dexamethasone reduces antioxidant concentrations [28], the significant rise in GSH concentration found in extract groups could indicate that the aqueous extract of *S. magnificum* has anti-free radical activity.

Hepatocyte cytolysis was found in the group of rats treated with dexamethasone alone and those administered with 400 mg/kg of the plant extract (but less marked) at the end of the subacute investigation. This result when combined with serum transaminase activities and relative liver weight, indicates dexamethasone-induced hepatic impairment. The kidney's histology revealed leukocyte infiltration and decreased glomerular cell density in rats treated with dexamethasone, while the other groups had normal renal parenchyma. Renal histology and uric acid concentration in the group of rats treated only with dexamethasone reflect the progressive deterioration of renal function, induced by dexamethasone. The pancreas was also examined histologically and it showed hypotrophy of the pancreatic islets in rats treated with dexamethasone only. This result confirmed the findings of Mawout *et al*. [19], that the endocrine pancreas is hypoactive in the presence of dexamethasone. Moreover, a non-significative (but notable) decrease of relative pancreas weight was noted in all dexamethasone-treated groups. Indeed, dexamethasone-induced insulin resistance results in hyperinsulinism which would lead to the fatigue of the Langerhans islets beta cells, inducing a decrease in the endocrine activity of the pancreas and hypotrophy of those islets. The absence or mild abnormalities in the liver, kidneys, and pancreas of rats who were given the aqueous extract of *S. magnificum* bark suggest that these organ tissues have been protected. These results were similar to those of Hasona *et al.* [25], who discovered that *Vitis vinifera* aqueous extract improved hepatic and renal impairment caused by dexamethasone. Flavonoids, anthocyanins,

saponins (secondary metabolites found in the extract) are recognized for their anti-diabetic and antioxidant properties, which could then be the active ingredients responsible for these results [29, 30, 31]

5. Conclusion

S. magnificum stem bark aqueous extract contained phenolic components, flavonoids, anthocyanins, saponins, gallic tannins, coumarins, and anthraquinones, according to qualitative phytochemical analysis. These compounds may be responsible for preventing hyperglycemia, oxidative stress, and histological damages, in the development of insulin resistance in the rat model. More research is needed to pinpoint the chemicals that cause these effects.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

Statement of ethical approval

The research was carried out following the guidelines of the Cameroon National Ethical Committee (Ref No. FW-IRB00001954, 22 October 1987). The study was carried out with the agreement of the Institutional Animal Ethics Committee.

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