Original

Maternal repercussions of the effect of *Chrysophyllum albidum* leaf extract on gestational diabetes mellitus rats induced by streptozotocin

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Abstract

Background: The study examined the effect of ethanolic leaf extract of Chrysophyllum albidum on gestational diabetes mellitus (GDM) in rats.

Method: 40 female oestrus rats and 20 male rats were caged for pregnancy; 30 pregnancies were recorded (GD1) and randomly divided into 6 groups of 5 rats each. Groups I - V were given 50 mg/kg of Streptozotocin by peritoneal injection to induce diabetes on the fourth day of pregnancy. Seventy-two hours later, fasting blood glucose levels > 200 mg/dl indicated GDM. Groups II, III, and IV received daily oral dosages of 250, 500 and 1000 mg/kg body weight of ethanolic leaf extract of Chrysophyllum albidum, respectively from GD7 to GD19, while group V got subcutaneously 0.3 IU/kg body weight of humulin. Group I was the GDM control and group VI was pregnant control. On GD19, blood samples were taken for hematological and biochemical analysis.

Result: Extract groups had blood glucose levels significantly ($P > 0.05$) improved, with a dose-dependent impact; superoxide dismutase increased, while malondialdehyde decreased. Total cholesterol, triglycerides, low-density lipoprotein were lowered, and high-density lipoprotein was increased. Alkaline phosphatase, aspartate transaminase, and alanine transaminase plasma levels all decreased. Albumin and total protein considerably increased ($P>0.05$), whereas creatinine and bilirubin decreased. WBC and monocytes decreased; also, RBC, lymphocytes, and neutrophils were unaffected. Hemoglobin level and Packed Cell Volume both increased, though the increase was not significant.

Conclusion: Study suggests that C. albidum have a protective effect on Streptozotocin-induced gestational diabetes, decreasing oxidative stress markers and diabetic complications.

Keywords: Chrysophyllum albidum, diabetic pregnancy, medicinal plant, antioxidant, Streptozotocin

Introduction

Diabetes mellitus is a chronic metabolic disorder resulting from a relative shortage of insulin and/or decreased insulin action.[1] Since 463 million people worldwide have diabetes and that number is expected to reach 700 million by the year 2045, there has been an increase in the prevalence of the disease in all geographical areas.[2]

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Gestational diabetes is any degree of glucose intolerance that occurs during pregnancy. It is characterized by pregnancy-related glucose intolerance, which can lead to a variety of issues for both the mother and the fetus, including hyperglycemia.[3] Up to 10% of expectant mothers experience gestational diabetes, which increases their risk of having chronic problems and comorbidities of diabetes while carrying a child.[4] Women with GDM have a range of treatment choices, including medical nutrition therapy, exercise, lifestyle and behavioral management, pharmacologic therapy, such as human insulin, and oral hypoglycemic drugs.[4] A growing fetus, however, is known to be impacted by some medications, either directly or through indirect effects. Therefore, despite tremendous progress in the discovery of drugs, diabetes remains presents a major global health problem.[5]

African nations are increasingly turning to medicinal plants to advance healthcare. One of the factors favoring an increase in the use of plants is the fact that their side effects are far less obvious than those of synthetic pharmaceuticals, which makes them particularly appealing to the populace. The benefits of medicinal plants have been assessed using a variety of metrics, including biochemical indicators, liver and kidney function tests, antioxidant status and more. Hence, a growing number of people are turning to herbal therapy as an alternative to cure a range of disorders including GDM.[6] As a result, the use of plant products with anti-diabetic effects has become more widespread. The use of medicinal herbs during diabetic pregnancy has received little research.[7] Hence, the safety and efficacy of herbal extract in pregnancy are now the subject of research.

Excessive oxidative stress has been connected to the pathogenesis of pregnancy with diabetes.[8]

Chrysophyllum albidum (*C. albidum*) Sapotaceae, known as Africa star apple have been known in Nigeria to have ethnomedicinal value and also considered to possess many different medicinal properties. *C. albidum* different parts have been reported to have antidiabetic effects such as the root bark,[9] seed,[10] fruit.[11] Idaguko et al. [12,13] reported that administering *C. albidum* leave extract to a diabetic rat model's boosted body weight, insulin, reduced glutathione (GSH), superoxide dismutase (SOD), Catalase (CAT), High density lipoprotein (HDL-C) and red blood cell levels while decreasing blood glucose, malondialdehyde (MDA), total cholesterol (TC), Triglyceride (TG), Low density lipoprotein (LDL-C)..

There are evidences that extracts of medicinal plants with different radical-scavenging properties could provide potent antioxidant defenses.[8] Since oxidative damage has been associated with the development of gestational diabetes issues.[14] Numerous plant extracts, such as *Artemisia dracunculus, Ginkgo biloba, Opuntia spp, Momordica charantia, Plantago ovata, Trigonella foenum-graecum, Allium sativum and Cinnamomum cassia* have been used to manage diabetes orally in folk medicine.[15] Pancreatic islet cells die as a result of streptozotocin (STZ), which is widely used in research to produce diabetes mellitus.[16] We predicted *Chrysophyllum albidum* could improve the maternal outcomes in experimental diabetic pregnancy. As far as we are aware, no studies have looked into how using this herb can affect diabetic pregnancies. We were inspired to use *C. albidum* extract in order to better comprehend the hematological and serum biochemical changes in pregnant rats with STZ-induced diabetes.

Method

Materials

Chemicals and reagents Streptozotocin were purchased from Sigma–Aldrich (St. Louis, MO, USA). Humulin 70/30 kits were purchased from the Diagnostic Company in Mushin. Lagos State (Nigeria).

Plant Collection and Extraction

The plant *Chrysophyllum albidum* was taken from Jesus the Saviour Monastery Elele in Rivers State, Nigeria. The leaf was identified and authenticated in the Department of Botany of the University of Lagos. The *C. albidum* leaves were cleaned, air dried for two weeks, cut into pieces by hand, and then coarsely powdered in an electric mill. The 70% of ethanol and the 760g of powdered leaves were mixed in a 1:10 powder to solvent ratio. The mixture was stirred before being allowed to stand for 24 hours. The mixture was filtered using No. 1 Whatman paper. The filtrate was then concentrated in a rotatory evaporator at a temperature of 40°C, and the concentrate was then further dried in a hot water bath at a temperature of 45°C. Until it was used in the experiment, the dried extract was kept in the refrigerator at a temperature of 4°C, sealed in a dry clean container.

Experimental Animals

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Wistar female rats (150-170 g, n=40) and male rats (180-200 g, n=20) were used for the experiment. The rats were obtained from the Animal House of the University of Port Harcourt and kept for the experiment in the Animal Facility of Madonna University Elele, Rivers State. The rats had a week to acclimate before the test. The rats were housed in a stainless-steel cage with 12-hour cycle of light and darkness. Throughout the test, rats were given a necessary meal (grower pelletized) and an unending supply of water.

Mating and induction of diabetes

The blood glucose levels were determined before the experiment commenced by using an Accu-check(R) Active glucose strips and test meter from Roche Diagnostic, Mannheim, Germany; the blood glucose level was expressed as mg/dl, and any blood glucose level greater than 80mg/dl in the rats were excluded. The estrous cycle was determined daily using the vaginal smear technique. The female rats were allowed to mate by putting two females in estrous with a male rat in the cage. Female rats were checked for the existence of a sperm plug after an overnight mating. To confirm pregnancy, a vaginal smear technique was used to check for the presence of spermatozoa. Gestational day 1 (GD 1) of pregnancy was observed as the day sperm were found in the vaginal smear. A total of thirty fertilized females were divided into six groups of five animals each using random distribution.

The animals were fasted for 12 hours prior to the GD4; and on GD 4, Streptozotocin (STZ) was injected intraperitoneally to induce diabetes (50 mg/kg diluted in sodium citrate buffer, 0.1 mol/L) on groups I, II, III, IV and V, except group VI that serves as the control. The diabetic condition was confirmed 72 h afterwards by measuring the fasting blood glucose (FBG) levels using Accu-Chek glucometer Roche Diagnostic, Mannheim, Germany. Rats were considered diabetic if their fasting blood glucose levels were > 200 mg/dl.[17] Additionally, to make Humulin 70/30 (0.3 IU/ kg bw subcutaneously (s.c)), 1ml of humulin was diluted in 10 ml of distilled water.[18] Before the study began, every week, during the treatment, and at the end of the study; the blood glucose levels were assessed. The rats were fed a standard rat chow diet and provided with water *ad libitum*. The *C. albidum* extract were administered once daily using oropharyngeal cannula and the humulin was administered subcutaneously on the rats thigh from gestation day 7 to gestation day 19; until the time the animals were sacrificed.

Group I: diabetic pregnant, giving normal saline;
Group II: diabetic pregnant received 250 mg/kg bw. of *C. albidum* extract
Group III: diabetic pregnant received 500 mg/kg bw. of *C. albidum* extract
Group IV: diabetic pregnant received 1000 mg/kg bw. of *C. albidum* extract
Group V: diabetic pregnant received humulin (0.3 IU/ kg bw)
Group VI: pregnant normal control.

Blood collection

The weights of the rats were checked before the commencement of the experiment, during and at end of the experiment. On GD19, the rats had blood drawn from their caudal vein, which was then applied to ACCU-Check strips. Blood samples for hematological and biochemical studies were taken from the retro-orbital sinus.

Antioxidant Enzymes Assay

Superoxide Dismutase (SOD) activity was determined by method described by [19]. Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method of [20].

Plasma Lipid Profile Determination

Plasma Total cholesterol (TC) levels, Plasma Triglyceride (TG), High Density Lipoprotein (HDL) were determined using a Randox diagnostic kit. [21, 22] Low Density Lipoprotein (LDL) was calculated using the empirical equation of [23]

Biochemical assays

The following parameters: bilirubin (Bil), total protein (TP), creatinine, albumin (ALB), alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined using assay kits.

Haematological estimation

The haematological parameters (red blood cell count (RBC), haemoglobin (Hb), packed cell volume (PCV), white blood cell count (WBC), and WBC differentials count (neutrophils, monocytes and lymphocytes) were analyzed according to the standard techniques described by [24, 25].

Statistical analysis

With the aid of the software GraphPad Prism 8.0.1 (GraphPad, San Diego, CA); data analysis was carried out. The Analysis of Variance (ANOVA) and Bonferroni post-test were used to evaluate all data.

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Results were given as mean SD and were deemed statistically significant at P <0.05.

Results

Blood glucose level

As shown in table 1, blood glucose levels of the rats of the non-diabetic pregnant group remained at approximately 78 mg/dL throughout the study. In the diabetic pregnant groups, blood glucose levels were above 200 mg/dL and following administration of *C. albidum* at doses of 250, 500 and 1000 mg/kg b. w, the blood glucose levels were significantly (P<0.05) reduced when compared to the non-diabetic pregnant control group VI. However, blood glucose in group I (diabetic pregnant) increased throughout the experiment.

Table 1: Effects of ethanolic leaf extract of *C. albidum* on fasting blood glucose level

Groups	Blood glucose levels (mg/dl) After induction of diabetes				
	Day 0	Day 1	Day 4	Day 10	Day 19
I	67.8±18.4	210.2±59.7	220.4±59.2^	233.4±58.8^	256.4±28.1^
II	89.8±6.6	218.0±59.7	192.8±74.9^	167.4±74.8^*	120.4±74.8^*
III	90.2±7.5	210.0±92.0	191.8±15.2^	154.2±76.5^*	114.2±76.5^*
IV	84.8±3.2	204.6±11.6	185.8±14.3^	145.0±12.5^*	99.0±12.5^*
V	78.0±11.9	213.6±61.1	187.6±70.8 ^	131.8±14.3^*	89.8±14.3*
VI	75.2±8.9	77.0±7.7	78.4±4.3	74.0±9.3*	74.0±9.3

Values are expressed in Mean ± SD, Number of animals = 5, *(p <0.05) significant difference when compared with group I, ^ (p<0.05) significant difference when compared with VI

Body weight and organs

At the end of the study, the diabetic pregnant (group I) rats had decrease in body weight which was significantly (P <0.05) decrease when compared to the non-diabetic pregnant control group VI. The diabetic pregnant rats treated with *C. albidum* (Groups II-IV) showed a significantly increase in their body weight in a similar manner with that of non-diabetic pregnant control rats (Group VI) (Figure 1). There was a decrease in the pancreatic weight in the diabetic pregnant group I, but it was not significant. However, there was no significant difference in the weights of the heart, spleen and kidney in all the groups. There was an increase in the weight in the liver of the diabetic pregnant group I but the increase was not significant (Table 2).

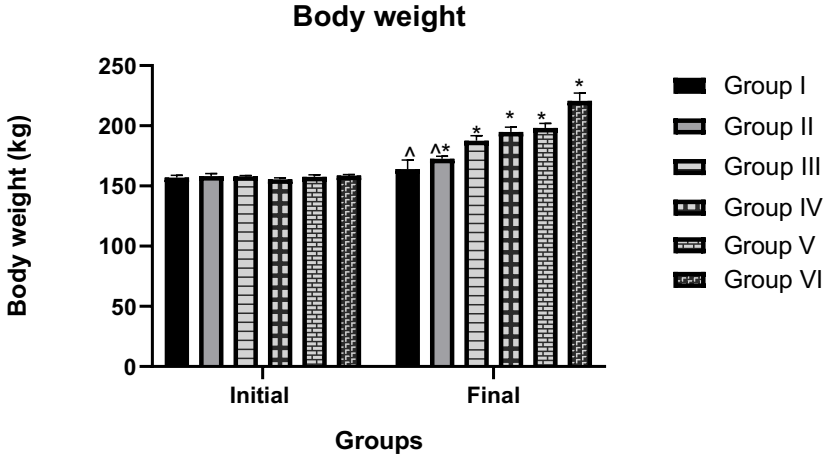

Figure 1 shows effect of *C. albidum* extract on initial and final body weight of experimental rats. Values are expressed as means ± SD (n= 5 rats). *p < 0.0001 compared to diabetic pregnant group I; ^ p < 0.05 compared to non- diabetic pregnant group VI. I = diabetes pregnant; II = diabetes pregnant + 250 mg/kg extract; III = diabetes pregnant + 500 mg/kg extract; IV = diabetes pregnant + 1000 mg/kg extract; V = diabetes pregnant + humulin (0.3UIL); VI= non-diabetic pregnant control.

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Table 2: Effect of ethanolic leaf extract of *C. albidum* on the organs weight

Groups	Liver	Heart	Kidney	Pancreas	Spleen
I	4.74±0.61	0.50 ± 0.03	0.51±0.07	0.30 ± 0.02	0.40±0.11
II	4.67 ± 0.37	0.50 ± 0.03	0.43±0.02	0.35 ± 0.02	0.47 ± 0.06
III	4.54 ± 0.6	0.40 ± 0.06	0.44±0.04	0.45 ± 0.08	0.54 ± 0.14
IV	4.47 ± 0.64	0.43 ± 0.02	0.43±0.03	0.40 ± 0.04	0.35 ± 0.04
V	4.33 ± 0.35	0.48 ± 0.03	0.46±0.04	0.48 ± 0.07	0.47 ± 0.04
VI	4.13 ± 0.35	0.51 ± 0.03	0.40±0.03	0.53 ± 0.19	0.61 ± 0.08

Values are expressed in Mean ± SD, Number of animals = 5.

Antioxidant status

There was a significant (p< 0.05) decrease in SOD activities and an increase in the Malondialdehyde (MDA) content in the diabetic pregnant group I when compared to non-diabetic pregnant group VI (control). The SOD activities in group V (humulin) and the treated groups (II, III and IV) showed an increase when compared to the non-diabetic pregnant group VI (control). Also, there was a significant (p< 0.05) decrease in MDA in all the treated groups, which was dose dependent when compared to the diabetic pregnant group I. (Figure 2).

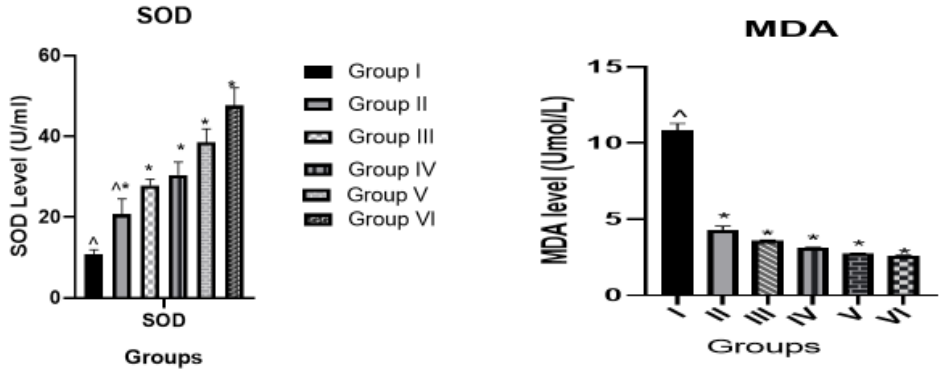

Figure 2 shows activities of superoxide dismutase (SOD) and Malondialdehyde (MDA); Values are expressed as mean ± SD. N= 5 in each group.*= significant difference when compared with group I (p<0.05). ^= significant difference when compared with VI (p<0.05)

Table 3 shows the lipid profile which was determined by serum levels of total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol (LDL-c). There was a significant (p< 0.05) increase in TC, TG, LDL-c (Groups II, III, and IV) and a significant decrease in HDL in the diabetic pregnant group 1. However, oral intake of *C. albidum* resulted in a significant (p<0.05) decrease in TC, TG, LDL-c (Groups II, III, and IV) and a significant increase in HDL when compared to the diabetic pregnant group 1.

Table 3: Effects of ethanolic leaf extract of *C. albidum* on lipid profile

Groups	Parameters			
	LDL(mg/dl)	HDL (mg/dl)	TG (mg/dl)	TC (mg/dl)
I	18.96 ± 0.21^	12.94 ± 1.98^	184.34 ± 0.86^	143.5 ± 12.3 ^
II	3.56 ± 0.35 *	20.03 ± 1.44 *^	129.07 ± 3.05^	114.6 ± 9.2*^
III	3.29 ± 0.07*	23.31 ± 0.67*^	109. 86 ± 3.56 *^	112.6 ± 1.5*^
IV	2.82 ± 0.27*	39.29 ± 4.15*	58.29 ± 4.53*	111.6 ± 5.4* ^
V	2.59 ± 0.24*	40.35 ± 1.02*	53.73 ± 2.32*	111.9 ± 9.2*
VI	2.25 ± 0.39*	35.62 ± 1.80*	42.44 ± 1.97*	81.3 ± 1.8*

Values are expressed as mean± SD. N= 5 in each group.* (p<0.05) significant difference when compared with I. ^(p<0.05) significant difference when compared with VI.

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The level of hepatic function activities of Alkaline phosphatase (ALP), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were shown, a significantly increased ($p<0.05$) was observed in diabetic pregnant group I, when compared to the non-diabetic pregnant control group VI. Moreover, treatment of diabetic pregnant rats with ethanolic leaf extract of *C. albidum* and humulin resulted in significant decrease in the levels of ALP, AST and ALT when compared to the diabetic pregnant group I. However, the levels of ALP, AST and ALT in the treated groups were dose dependent (Table 4).

Table 4: Effects of ethanolic leaf extract of *C. albidum* on liver function enzymes

Groups	ALP (U/L)	AST (U/L)	ALT (U/L)
I	139.20 ± 26.07^	179.02 ± 27.29^	52.66 ± 3.72^
II	89.49 ± 26.54*	145.82 ± 15.16* ^	29.42 ± 0.77*^
III	80.69 ± 29.50*	121.82 ± 48.37*^	27.12 ± 0.31*^
IV	78.18 ± 26.49*	76.33 ± 12.60*^	19.78 ± 1.76*^
V	76.37 ± 21.22*	60.38 ± 12.79*^	14.12 ± 0.31*
VI	70.37 ± 14.96 *	42.96 ±11.07*	12.44 ±1.97 *

Values are expressed as mean± SD. N= 5 in each group.* ($p<0.05$) significant difference when compared with group I ($p<0.05$). ^ ($p<0.05$) significant difference when compared with group VI.

The results in table 5 showed that there was a reduced levels of serum total protein and albumin concentrations in untreated diabetic pregnant rats when compared with the non-diabetic pregnant control group VI. The ethanolic extract of *C. albidum* and humulin showed an increase protein concentration and albumin to near normalcy as observed in the non-diabetic pregnant control VI. However, the untreated pregnant diabetic rats exhibited significant increase ($P < 0.05$) in activities of bilirubin and creatinine when compared to the non-diabetic pregnant control VI. Continuous administration of ethanolic extract of *C. albidum* to the diabetic pregnant rats' groups (II, IV and V) was able to restore the bilirubin and creatinine back to normalcy when compared to non-diabetic pregnant control VI.

Table 5: Effects of ethanolic leaf extract of *C. albidum* on kidney function tests

Groups	Albumin (mg/dl)	Creatinine (mg/dl)	Bilirubin (mg/dl)	Total Protein (g/dl)
I	0.70±0.03^	4.78±0.01^	1.52±0.01^	7.08±0.00^
II	1.61±0.57*	0.31±0.02*	0.50±0.00*	10.08±0.10
III	1.51±0.85*	0.25±0.02*	0.58±0.01*	11.27±0.43
IV	1.50±0.04*	0.22±0.11 *	0.60±0.00*	11.52±0.10*
V	2.00±0.03*	0.16±0.54*	0.62±0.01*	12.00±0.42*
VI	2.44±0.01*	0.14±0.02*	0.51±0.00*	12.17±0.65*

Values are expressed as mean± SD. N= 5 in each group. *($p<0.05$) significant difference when compared with group I. ^ ($p<0.05$) significant difference when compared with group VI

The untreated diabetic pregnant group I showed a decreased in RBC, PCV and HB when compared to the non-diabetic pregnant control group VI. While treatment with *C. albidum* extract showed no significant change in RBC, PCV, HB when compared to the non-diabetic pregnant control group VI. Neutrophil and monocytes was increased in the untreated diabetic pregnant rats' group I, but it was not significant when compared to the extract treated groups (II, III and IV). However, lymphocytes were decreased in the diabetic pregnant group I, but it was also not significant when compared to the extract treated groups. WBC levels significantly ($P<0.05$) increase in the untreated diabetic pregnant group I when compared with the extract's groups (II, III, and IV) and pregnant control group VI. (Table 6).

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Table 6: Effects of ethanolic leaf extract of *C. albidum* on hematological parameters

Parameters	I	II	III	IV	V	VI
RBC (×10/mm3)	5.94±0.09	9.17±0.19*	8.29±0.04*	8.32±0.35*	8.32±0.38*	8.32±0.37*
PVC(%)	37.67±3.58	41.00±0.92*	43.33±0.37*	47.50±0.67*	45.00±0.84*	46.50±0.22*
HB(g/dl)	10.57±1.18	14.55±0.29*	15.00±0.45*	15.80±0.22*	15.15±0.07*	16.500±0.09*
WBC (x10^{14}/L)	20.99±0.07^	4.41±0.35*	4.63±0.15*	4.11±0.26*	4.39±0.05*	3.35±0.65*
Neutrophil(%)	61.33±1.8	57.00±2.22	55.00±0.89	58.00±0.00	55.00±1.34	58.50±1.57
Monocyte(%)	2.33±0.21	1.33±0.00	1.70±0.22	1.50±0.24	1.50±0.21	1.40±1.00
Lymphocyte(%)	39.33±0.21	40.67±3.12	42.00±0.00	41.00±0.45	42.50±0.22	42.50±0.24

Values are expressed as mean± SD. N= 5 in each group. *($p<0.05$) significant difference when compared with group I. ^ ($p<0.05$) significant difference when compared with group

Discussion

Studies show that streptozotocin can cause the islets of Langerhans to have a huge selective death and reduction of beta cells, which results in a partial or whole loss of insulin synthesis and hyperglycemia.[26] Humulin, an anti-diabetic drug, is produced as a synthetic replica of human insulin. Humulin helps the pancreatic islet cells secrete insulin, which lowers blood sugar by promoting the uptake of glucose into skeletal muscle and fat balance.[27] Most of the body's glucose is stored in the skeletal muscles and liver, and in order to prevent chronic hyperglycemia and conserve the energy in form of glycogen to meet future requirements, the amount of glucose in cells is tightly regulated.[28]

As a result of gestational diabetes mellitus, the bodily tissues are unable to use the glucose, which increased protein usage and decreased body weight.[14] The increased body weight reported in this study after receiving *C. albidum* leaf extract could be due to higher production of structural protein. According to Abu-Odeh and Talib[29] one potential cause of body weight growth in rats given plant extracts may be an increase in pancreatic activity, which may have helped the tissues to utilize glucose.

The reduction or absence of insulin synthesis by the pancreatic beta cells, may have been caused by beta cells destruction, resulting in the rise in fasting blood glucose levels of the diabetic rats in this study. This is in line with the conclusions of [30], who linked beta cell degeneration to the rise in fasting blood glucose levels in diabetic rats. The groups treated with 250, 500, and 1000 mg/kg of the *C. albidum* extract showed a significant reduction in glucose levels, indicating that the leaf extract has hypoglycemic effect. Significant antidiabetic efficacy in rats was also achieved by other *Chrysophyllum* species.[31] Previous research conducted in our lab has demonstrated that *C. albidum* lowers blood glucose levels by boosting pancreatic insulin production and enhancing glucose absorption.[12]

Oxidative stress is exacerbated, and the antioxidant state is weakened by gestational diabetes mellitus according to [32]. Diabetes brought on by STZ worsens the state of antioxidant enzymes and increases lipid peroxidation.[33] Superoxide dismutase (SOD) is the sole enzyme in the enzymatic antioxidant defense system that takes superoxide anions as a substrate and produces hydrogen peroxide and oxygen as a metabolite, reducing reactive oxygen species' (ROS') harmful effects.[34] In pregnant diabetic rats, increasing lipid peroxidation may have caused tissue damage and a breakdown in the antioxidant defense systems as indicated by increased malondialdehyde (MDA) levels and decreased SOD activity. Increased Malondialdehyde (MDA) levels and reduced SOD activity in pregnant diabetic rats suggest that lipid peroxidation was elevated, which may lead to tissue damage and a breakdown in the antioxidant defense mechanisms. As a result, treatment with the ethanolic extract of *C. albidum* significantly increases SOD activity and decreases MDA. Thus, *C. albidum* may possess antioxidant properties and protect tissue against lipid peroxidation. Similar report has been document of the root back of *C. albidum* on SOD and MDA.[35]

According to research, diabetes causes alterations in the haematological pathways, and several changes affecting RBCs and WBCs.[36] Additionally, it has been demonstrated that hyperglycemia impairs RBC deformability by increasing the generation of ROS, which in turn increases RBC haemolysis.[37] The non-enzymatic glycosylation of proteins on the erythrocyte membrane, the cross-linking of membrane lipids, and the inactivation of RBC antioxidant enzymes like SOD are all brought on by increased ROS production.[38] SOD helps to maintain RBC's longevity and preserve haemoglobin in a reduced oxygen-binding state while also limiting oxidative alterations to membrane lipids,

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structural proteins, and metabolic enzymes.[39] *C. albidum's* ability to lower blood glucose and boosts antioxidant status may be the cause of its ability to considerably improve RBC indicators.

An elevated WBC count is a sign of inflammation and is related to how well the body can fight against illnesses.[40] The body's response to foreign substances and hyperglycemia-induced oxidative stress, which change normal physiological processes, are the causes of the observed rise in WBC count in the untreated diabetic pregnant rats as reported in this study. However, the reduction in WBC count upon treatment of diabetic pregnant rats with *C. albidum* leaf extracts may be attributable to its antioxidant effect and free radical scavenging activity and anti-inflammatory action,[41] thereby indicating a boost in the immune system. Lymphocytes are crucial for maintaining the body's immune system and defense, the drop in lymphocytes may be a reaction to the stress caused by diabetes mellitus.[42] In a diabetic state, the immune system may be weakened, and the body may be more susceptible to infections; as the danger of infection is higher in diabetics, the increase in neutrophils may be attributable to their participation in phagocytic cellular processes against various antigens.[43, 44]

Pregnant women with GDM have aberrant changes to their lipid profiles; and gestational diabetes is also a risk factor for cardio metabolic diseases of the mother and offspring.[5, 45] The significant hyperlipidemia that characterizes diabetes may be viewed as the result of the unrestricted activities of lipolytic hormones on the fat depots.[46] When plasma cholesterol circulates in the blood, it is mostly carried by low-density lipoprotein cholesterol (LDL-c). LDL-c can gradually accumulate in the inner walls of arteries, where it forms plaque that narrows the arteries and causes arthrosclerosis.[47] High-density lipoprotein cholesterol (HDL-c) is thought to have anti-atherogenic characteristics. Therefore, tend to transfer cholesterol atheroma from arteries back to the liver for excretion or reutilization. Furthermore, it has been demonstrated that a rise in HDL-c correlates negatively with coronary heart disease; consequently, having high levels of HDL-c helps prevent cardiovascular disease.[48] Triacylglycerol (TG) is the primary form of fatty acid storage, and a high triglyceride level is a hallmark of diabetic dyslipidemia.[49] It is well known that TG regulate interactions between lipoproteins. Evidence shows that TG levels and the TG/HDL ratio can serve as both a reliable indicator of GDM risk as well as a potential risk factor for developing of type 2 diabetes.[45] According to the study, the untreated diabetic pregnant group showed higher levels of TG, TC, LDL, and lower levels of HDL. However, the lipid profile of diabetic pregnant rats treated with the *C. albidum* extract improved by lowering LDL-c, TC, TG and raising HDL-c. Thus, by reducing and regulating the lipid profile, the leaf extract of *C. albidum* may have demonstrated its antihyperlipidemic effect.

Since the mitochondria and cytoplasm of hepatocellular cells contain the most accurate hepatic damage markers, an increase in ALT activity is indicative of hepatocellular damage and is also frequently accompanied by increase in AST.[50] In a recent study, elevated ALT and AST were connected to gestational diabetes.[51] Therefore, it is believed that a rise in the plasma activity of these enzymes is a reliable indicator of liver damage. According to Begum and Shanmugasundaram,[52] these enzymes directly contribute to the increased synthesis of keto acids in diabetes conditions. According to the study's findings, the diabetic pregnant group was substantially related with an increase in the liver enzymes (ALT, AST and ALP) as compared to the non-diabetic pregnant control group I. Therefore, the plant may contain compounds that have hepatoprotective actions because the treatment of streptozotocin-induced diabetic pregnant rats with extract of *C. albidum* leaves decreased the levels of AST, ALP, and ALT activities.

Bilirubin is a pigment that is produced as a result of the breakdown of heme in red blood cells and is carried to the liver where it is secreted into the bile by the liver.[53] Bilirubin is a pigment that shows the cellular integrity of the liver. The dosage of *C. albidum* extract caused a considerable drop in bilirubin levels. The extracts may have decreased the danger of liver damage; as a result, may have improved the bilirubin status. Creatinine is a marker coupled with electrolyte balance when assessing the activities of the liver in diabetic condition. Consequently, excessively high levels of creatinine in the blood are a marker of impaired kidney function and may indicate renal failure.[54] This study shows that untreated diabetic pregnant rats had elevated serum creatinine levels. However, the levels of creatinine were significantly reduced in the diabetic pregnant rats treated with *C. albidum*. Due to *C. albidum* extracts' power to lower blood sugar, which may have enhanced the kidneys' ability to remove these waste products from the blood and so maintained the kidney. A significant decline in the total protein of the liver's contents is an indication of liver toxicity, but a rise in tissue serum total

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protein is a reflection of all plasma proteins in the blood and also a marker of tissue damage.[55] Albumin is the primary protein in plasma, thus anything that lowers albumin levels is likely to have a detrimental impact on all other proteins as well. Hence, an increase in total protein is often caused by tissue injury.[56] A disturbed glucagon-mediated regulation of cyclic AMP production in insulin deprivation may be the cause of the reported decrease in the total protein content in STZ-induced untreated diabetic pregnant rats. As a result, giving diabetic pregnant rats an ethanolic extract of *C. albidum* dramatically reduced the proteolysis brought on by insulin shortage and raised the level of plasma proteins and albumin to levels that were close to normal. This implies that the *C. albidum* may have improved the liver and kidney's functional state, as suggested by this study. As a result, the extract can significantly repair tissue damage brought on by STZ in rats.

Strength and limitation of the study

Since the pathogenic features of the created GDM rat model were comparable to those of GDM patients in humans, we utilized rats as the perfect model organism. Furthermore, the study's limitations may include the inability to determine the fetus's fate due to the study's design, which limited it to the gestational period.

Implications of the findings

The findings suggest that *C. albidum* may be utilized to treat GDM and its complications, because it has been demonstrated to alleviate complication that results from the disease.

Conclusion

According to the research, STZ-induced diabetic pregnant experimental rats showed hypoglycemia and antioxidant activities when given an oral dose of an ethanolic extract of *C. albidum*. The outcomes also demonstrated the extract's benefits in lowering the imbalanced lipid metabolism brought on by diabetes. Therefore, it may be inferred from this research that in addition to its hypoglycemic and antioxidant effects, the ethanolic leaf extract of *C. albidum* may also protect the liver, kidney, and blood from harm caused by diabetes.

Declarations

Ethical consideration: All the experimental procedures and protocols used in this study were approved by the Committee for Ethics in Animal Experimentation of Madonna University Elele. Rivers State, in accordance with the National Institution of Health Guideline Principles of Laboratory Animal in Biomedical Research [57]

Authors' contribution: Conception and design of the research: Chika Anna Idaguko and Osagie Mike Odigie. Conducted the research: Mariafaustina Nku-ogabi, Godwin Sunday Nduohosewo, Chiamaka Celestina Okpara-Nkonneh, Onah Alexis, Lekam Esther Patrick. Analysis and interpretation of data: Chika Anna Idaguko and Osagie Mike Odigie. All authors were involved at different stages of writing the manuscript.

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