



Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: <http://www.elsevier.com/locate/jtcme>

Original Article

Chromatographic fractions from *Chrysophyllum albidum* stem bark boost antioxidant enzyme activity and ameliorate some markers of diabetes complications

Babalola Ola Yusuf ^{a, b, *}, Musa Toyin Yakubu ^b, Musbau Adewumi Akanji ^b^a Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria^b Department of Chemical Sciences, College of Natural and Applied Sciences, Summit University Offa, Nigeria

ARTICLE INFO

Article history:

Received 11 May 2020

Received in revised form

12 January 2021

Accepted 16 January 2021

Available online 21 January 2021

Keywords:

Alloxan

Dyslipidemia

Glycosylated haemoglobin

C-reactive protein

Chrysophyllum albidum

ABSTRACT

Background and aim: Diabetes, with hyperglycaemia as hallmark, is a global crisis that reduces the antioxidant status and produces complications when poorly managed. The development of complications can be indicated by inflammation, lipid peroxidation and the accumulation of glycation adducts. Thus, the attenuation of hyperglycaemia and boosting of antioxidants status is key in ameliorating markers of diabetes complications. This work evaluated the potency of *Chrysophyllum albidum* stem bark on some markers of diabetes complications.

Experimental procedure: A total of 100 female rats (180.80 ± 8.50 g) were assigned into ten groups of ten animals each; control received 1.0 ml of distilled water while those in groups DC, RD, F1, F3, F4, F5, F7, F9, F10 were induced into diabetes by intraperitoneal administration of 120 mg/kg body weight of alloxan and were orally administered distilled water, glibenclamide, 2.5 mg/kg of the chromatographic fractions 1, 3, 4, 5, 7, 9, and 10 respectively, once daily for 14 days. F7 was profiled for its bioactive constituents and the pancreas histology of the rats were examined.

Results: Chromatographic fractions F5 and F7 significantly decreased fasting blood glucose, glycosylated haemoglobin, C-reactive protein, total cholesterol, triglycerides, atherogenic index, malondialdehyde while insulin, high density lipoprotein, catalase, superoxide dismutase activities significantly increased. Fraction F7 revealed eight compounds and restored the distorted pancreas.

Conclusion: Fraction F7 ameliorated the markers of diabetes complications considered in this study better than F5, restored the compromised pancreas and can be explored as lead candidate for production of drug for the management of diabetes.

© 2021 Center for Food and Biomolecules, National Taiwan University. Production and hosting by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

The incidence of diabetes is wide-reaching with an increasing morbidity and mortality associated with the development of diabetes complications arising from oxidative stress and inflammation¹ among other factors. Like other diseases, diabetes and its complications have been linked to free radical generation with glucose autoxidation as a major source of free radicals in chronic

hyperglycemia.² Free radical production is important in normal cellular homeostasis and in the bodies response to pathogens though, many of the diabetes complications result from excessive free radical generation and oxidative stress.¹

Oxidative stress and inflammation that emanated from persistent hyperglycaemia are important drivers of these diabetes complications.³ The available orthodox drugs for the management of diabetes however, have various hitches.⁴ The attention now is in the direction of specific tactics that can attenuate both oxidative stress and inflammation to improve the disease burden. Biomarkers for envisaging the incidence and progress of the disease may therefore offer benefits in terms of early diagnosis and intervention.

This has triggered a search for a potential alternative from plants with little or no side effects that can be used to attenuate the

* Corresponding author. Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria.

E-mail address: ybabalola16@summituniversity.edu.ng (B.O. Yusuf).

Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

List of abbreviations

NDR	Non Diabetic Rats
DC	Diabetic Control
RD	Reference Drug
FBG	Fasting Blood Glucose
MDA	Malondialdehyde
SEM	Standard Error of Mean
TG	Triglycerides
LDL	Low Density Lipoprotein
VLDL	Very Low Density Lipoprotein
AI	Atherogenic Index

markers of diabetes complications. One of such plants commonly used in the Nigerian traditional medical practice for the management of diabetes is *Chrysophyllum albidum*.

Chrysophyllum albidum parts have been reported for various pharmacological actions. The root bark has been testified to have antihyperglycaemic, hepatoprotective and free radical scavenging activities.⁵ The seed cotyledon has hypoglycemic, hypolipidemic while the leaf has antioxidant activity.⁶ Also, the root, stem and seed cotyledon exhibited antimicrobial activity.⁷ Aqueous extract of *C. albidum* stem bark at 25 mg/kg body weight has been reported to have anti-diabetic activity.¹⁰ Previous investigation of the chemical constituents of *C. albidum* stem-bark revealed stigmasterol, epicatechin, epigallocatechin and procyanidin B5.¹¹ However, there is paucity of information on potentials of chromatographic fractions of *C. albidum* stem bark in management of diabetes and attenuating markers of diabetes complications and hence the need for this study.

2. Materials and methods

2.1. Plant material and authentication

Chrysophyllum albidum was obtained from a single matured plant in March 2016, from Ganmo in Ifelodun Local Government Area of Kwara State. Authentication was done at the Herbarium of Plant Biology, University of Ilorin, Nigeria. A voucher number UILH/001/1170 was assigned and a voucher specimen deposited.

2.2. Experimental animals

One hundred albino rats (180.60 ± 8.50 g) was obtained from the Animal House of the Department of Biochemistry, University of Ilorin, Ilorin, Kwara State, Nigeria.

2.3. Drugs, assay kits, and chemicals

Glibenclamide used was a product of Medico Remedies Pvt Ltd Juhu, Mumbai, India. Assay kits for C-reactive protein was a product of Bioway Chemistry Reagent Series, Jiang Ning District, Nanjing, China while glycosylated Hb was a product of Fortress Diagnostics Limited, Unit 2C Antrim Technology Park, Antrim, United Kingdom. Alloxan monohydrate was a product of Sigma Chemical Company, St. Louis, Mo, USA.

2.4. Methods

2.4.1. Preparation of the chromatographic fractions of *Chrysophyllum albidum* stem bark

The method described by Yakubu et al.¹² was adopted with some

modifications. The plant stem bark was cut into pieces, and oven dried at 40 °C (to protect against heat related damages to the phytochemicals) to a constant weight. The dried stem bark of *C. albidum* (2000 g) was extracted in distilled water (5 LL) for 72 h at 25 °C. This was then filtered using Whatman no 1 filter paper. The filtrate was freeze-dried (Labconco Freeze Drier, Model 64,132, Kansas City, Missouri, USA) to give 75.2 g (3.76% yield). This was then partitioned using ethyl acetate and n-butanol adopting the procedure of Muhit et al.¹³ Briefly, 20 g of the lyophilized aqueous extract was dissolved in 10 ml distilled water and then partitioned in 200 ml of ethyl acetate, and placed on electronic shaker for 45 min after which it was poured into a standing separating funnel and allowed to separate into two distinct phases-the supernatant which is the ethyl acetate-partitioned fraction, and the sediment. The procedure above was repeated before the sediment was extracted in n-butanol. The resulting solvent-partitioned fractions were concentrated using rotary evaporator to give ethyl acetate-partitioned fraction, n-butanol partitioned fraction and the residue. The fractions and the residue were kept in a desiccator, over silica gel, to ensure complete elimination of water before being used for *in vitro* and *in vivo* studies. Ethyl acetate partitioned extract was further subjected to chromatography using gradient elution method. The ten fractions obtained was then pooled to seven fractions (F1, F3, F4, F5, F7, F9, F10) based on their R_f values.

2.4.2. Ethical clearance

The study was carried out after ethical approval from the University of Ilorin Ethical Review Committee (UERC) with approval number UERC/ASN/2017/907 dated June 12th 2017.

2.4.3. Animal grouping and extract administration

A total of one hundred female rats were used in this study. The animals were randomly selected into ten groups of ten animals each with administration done once daily for fourteen days as follows:

Group	Treatment
Control	Non-diabetic rats +1 ml of distilled water
DC	Diabetic rats + 1 ml of distilled water
RD	Diabetic rats +2.5 mg/kg b.wt of glibenclamide
F1	Diabetic rats +2.5 mg/kg b.wt of F1
F3	Diabetic rats +2.5 mg/kg b.wt of F3
F4	Diabetic rats +2.5 mg/kg b.wt of F4
F5	Diabetic rats +2.5 mg/kg b.wt of F5
F7	Diabetic rats +2.5 mg/kg b.wt of F7
F9	Diabetic rats +2.5 mg/kg b.wt of F9
F10	Diabetic rats +2.5 mg/kg b.wt of F10

2.4.4. Induction of diabetes

After an overnight fast of the animals, diabetes was induced using the method described by¹⁴ with slight modification. The rats were induced into diabetes by a single intraperitoneal injection of 120 mg/kg body weight alloxan monohydrate. The rats were then orally administered 2 ml of 5% dextrose saline to overcome the initial alloxan-induced hypoglycaemia. Blood glucose levels of the rats were determined 72 h after alloxan administration. Blood samples were drawn from the sharply cut tail vein of the rats and placed on the test strip that had been inserted into the glucometer.¹⁵ Animals with blood glucose level equal to or more than 250 mg/dL were considered diabetic and used for the experiment.¹⁶

2.4.5. Preparation of serum and determination of biochemical parameters and pancreas histology

The procedure described by Yakubu et al.¹² was adopted for preparation of serum. Briefly, the blood was collected by cutting the jugular vein and the blood drained into sample bottles (plain and anticoagulant). Blood samples in the plain sample bottles were left undisturbed at 25 °C for 30 min to form clot after which the samples were centrifuged at 1282×g for 5 min. After centrifugation, the sera were collected by a means of Pasteur pipette into clean sample bottle, appropriately labeled and stored in freezer at 4 °C not later than 72 h of preparation for the biochemical assays.

Fasting blood glucose was determined as described by Yakubu and Ogunro.¹⁵ Serum insulin as described in the ELISA kits (Elabscience Biotechnology Co., Ltd), superoxide dismutase as described by Misra and Fridovich,¹⁷ Catalase as described by Beers and Sizer,¹⁸ malondialdehyde levels as described by Varshney and Kale,¹⁹ glycosylated haemoglobin as described by Trivelli et al.,²⁰ C-reactive protein as described by Burtis et al.,²¹ total cholesterol as described by Roeschlau et al.,²² triglyceride as described by Trinder,²³ high density lipoprotein-cholesterol (HDL-C) as described by Lopes-Virella,²⁴ low density lipoproteins (LDL-c) and very low density lipoproteins (VLDL-c) described by Friedewald et al.²⁵ while histology was done as described by Drury and Wallington.²⁶

2.5. Data analysis

Data were expressed as the mean ± SEM of ten determinations. Data were analyzed using one-way analysis of variance followed by Tukey's *post-hoc* test for multiple comparisons. Statistical significance was set at 95% confidence interval ($p < 0.05$) and graph Pad Statistical Package version 6.0 was used for the statistical analyses.

3. Results

3.1. Antidiabetic constituents in the chromatographic fraction F7 obtained from ethyl acetate-partition fraction of *Chrysophyllum albidum* stem bark

HPLC analysis of the F7 revealed eight compounds. Among the chemical constituents identified, stigmaterol had the highest peak with a retention time of 1.300 min whereas eleagnine had the lowest peak with retention time of 1.066 min (Table 1).

3.2. Fasting blood glucose and serum insulin levels of diabetic rats orally administered chromatographic fractions of *Chrysophyllum albidum* stem bark for 14 days

Compared with the control group, alloxan administration significantly ($p < 0.05$) increased the FBG level of the rats (Table 2). Among the seven column chromatographic fractions (F1, F3, F4, F5, F7, F9 and F10) administered to the diabetic groups at 2.5 mg/kg

Table 1

Chemical constituents of the chromatographic fraction (f7) obtained from ethyl acetate-partitioned fraction from *Chrysophyllum albidum* stem bark.

Compounds	Retention Time (min)	Concentration (ppm)
Eleagnine	1.066	1.450
Stigmaterol	1.300	2.267
Catechin	1.850	9.551
Epicatechin	2.183	7.319
Epigallocatechin	3.050	5.800
Hexadecane	3.450	0.177
Octadecanoic acid	4.000	1.227
Eicosane	5.316	6.210
Procyanidin B5	6.200	3.655

body weight, only F5 and F7 significantly ($p < 0.05$) reduced the FBG levels of their respective groups. By day 10, the F5 treated group was not significantly ($p < 0.05$) different from the glibenclamide-treated diabetic rats but did not compared well with the control group while F7 was not significantly ($p < 0.05$) different from the control group and compared better than the glibenclamide-treated diabetic rats. Other fractions were not significantly ($p < 0.05$) different from the diabetic control group.

Alloxan administration significantly ($p < 0.05$) decreased the serum insulin level when compared with the control group (Fig. 1). Administrations of the chromatographic fractions significantly ($p < 0.05$) increased serum insulin of the F7 treated diabetic rats only. The F5 treated group was not significantly ($p < 0.05$) different from diabetics receiving distilled water.

3.3. Effect of chromatographic fractions of *Chrysophyllum albidum* stem bark on antioxidant enzymes and malondialdehyde levels of alloxan-induced diabetic rats

Alloxan administration significantly ($p < 0.05$) increased the malondialdehyde (MDA) level and decreased the catalase and superoxide dismutase (SOD) activities (Table 3). Oral administration of chromatographic fractions of ethyl acetate-partitioned from *Chrysophyllum albidum* stem bark significantly ($p < 0.05$) reduced the MDA level and increased the SOD and catalase activities of the treated rats.

The MDA level of F5, F7 and the glibenclamide-treated diabetic rats were not significantly ($p < 0.05$) different from the control group. Catalase and SOD activities of the F7 treated diabetic rats were not significantly ($p < 0.05$) different from the control group while SOD activity of F5 treated diabetic rats was not significantly ($p < 0.05$) different from the glibenclamide-treated diabetic rats.

3.4. Glycosylated haemoglobin and CRP of diabetic female rats orally administered chromatographic fractions of *Chrysophyllum albidum* stem bark

Alloxan administration significantly ($p < 0.05$) increased the glycosylated haemoglobin (Fig. 2) and serum C-reactive protein level (Fig. 3) of the rats when compared with the control group. Administration of chromatographic fractions of the extract significantly ($p < 0.05$) decreased the alloxan-induced increase in glycosylated haemoglobin and serum CRP level though, only F7 treated diabetic rats was not significantly ($p < 0.05$) different from the control group.

3.5. Serum lipids of alloxan-induced diabetic rats orally administered ethyl acetate chromatographic fractions of *Chrysophyllum albidum* stem bark

Compared with the control group, alloxan administration significantly ($p < 0.05$) increased the serum cholesterol, triglycerides (TG), low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C), atherogenic index (AI) and significantly ($p < 0.05$) decreased the high density lipoprotein-cholesterol (HDL-C) (Table 4). Oral administration of both F5 and F7 significantly ($p < 0.05$) increased HDL-C and decreased other parameters. The serum triglycerides, low density lipoprotein-cholesterol, very low-density lipoprotein-cholesterol, atherogenic index and high-density lipoprotein-cholesterol of both fractions were not significantly ($p < 0.05$) different from the control group. Where:

Pancreas Histology of Alloxan-Induced Diabetic Rats Administered Chromatographic Fractions of *Chrysophyllum albidum* Stem Bark.

Table 2
Fasting blood glucose of female diabetic rats orally administered chromatographic fractions of *Chrysophyllum albidum* stem bark.

Day/Group	0	1	4	7	10	14
Control	59.29 ± 5.12 ^a	57.43 ± 2.82 ^a	59.43 ± 2.82 ^a	57.86 ± 4.41 ^a	55.14 ± 5.73 ^a	59.14 ± 3.18 ^a
DC	56.86 ± 5.01 ^a	383.43 ± 98.42 ^b	401.00 ± 93.79 ^b	410.14 ± 78.04 ^b	442.43 ± 70.30 ^b	499.14 ± 57.61 ^b
RD	57.43 ± 3.82 ^a	401.00 ± 96.09 ^b	316.71 ± 96.95 ^b	172.43 ± 27.04 ^c	83.57 ± 13.21 ^c	64.86 ± 9.84 ^a
F1	58.57 ± 5.53 ^a	384.43 ± 94.01 ^b	353.57 ± 89.35 ^b	330.43 ± 88.86 ^b	286.57 ± 76.77 ^d	253.00 ± 53.46 ^c
F3	57.00 ± 5.10 ^a	361.43 ± 83.97 ^b	338.43 ± 83.79 ^b	306.14 ± 67.52 ^b	276.57 ± 60.91 ^d	244.86 ± 49.03 ^c
F4	58.43 ± 6.70 ^a	351.86 ± 92.80 ^b	330.00 ± 99.13 ^b	317.14 ± 97.51 ^b	285.43 ± 97.46 ^{b,d}	240.14 ± 89.49 ^c
F5	56.29 ± 3.77 ^a	356.43 ± 101.17 ^b	300.86 ± 65.92 ^b	219.29 ± 15.12 ^d	99.57 ± 14.14 ^c	64.14 ± 12.25 ^a
F7	59.86 ± 3.80 ^a	396.57 ± 67.91 ^b	231.43 ± 41.13 ^c	142.86 ± 17.99 ^c	58.00 ± 9.26 ^a	57.14 ± 4.78 ^a
F9	58.86 ± 4.30 ^a	355.86 ± 54.80 ^b	323.86 ± 52.16 ^b	295.86 ± 50.33 ^b	262.29 ± 49.06 ^d	245.57 ± 45.54 ^c
F10	60.86 ± 4.85 ^a	409.86 ± 68.89 ^b	367.86 ± 65.76 ^b	331.43 ± 58.65 ^b	306.71 ± 53.05 ^d	279.86 ± 49.10 ^c

Values are mean of ten replicates ± SEM.
Values with different superscripts are significantly different from others (p < 0.05).

Where:
Control = non-diabetic rats receiving 1 ml of distilled water; DC = diabetic rats receiving 1 ml of distilled water; RD = diabetic rats receiving standard drug; F1-10 = diabetic rats receiving chromatographic fractions.

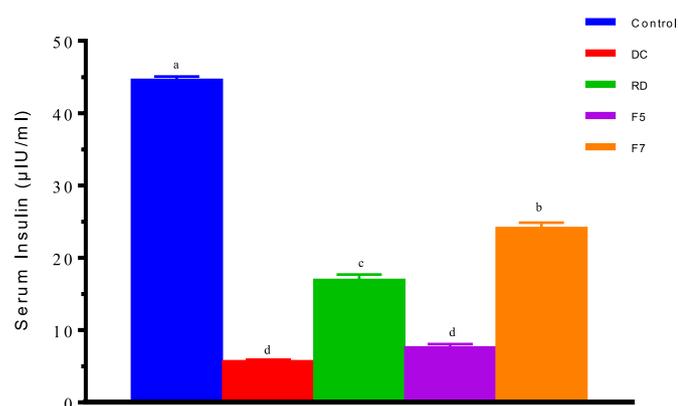


Fig. 1. Serum insulin of alloxan-induced diabetic rats orally administered chromatographic fractions of *Chrysophyllum albidum* stem bark for 14 days.

Where:
Control = non-diabetic rats receiving 1 ml of distilled water; DC = diabetic rats receiving 1 ml of distilled water; RD = diabetic rats receiving standard drug; F5 = diabetic rats receiving chromatographic fraction 5; F7 = diabetic rats receiving chromatographic fraction 7

The cross section of the pancreas of the control, reference drug (RD) and the F7 treated groups appeared normal with no visible lesion seen whereas the diabetic control (DC) rat revealed parenchymatous portion of acini and islet portions that are completely obliterated (acini and islet not seen in the section), loose connective tissue and adipose tissue seen (long arrows) and they are highly infiltrated by inflammatory cells (short arrows); scale bar: 50 µm (Plate 1). The pancreas of the F5 treated group revealed a severe congestion of the blood vessels (black arrows). The parenchyma is almost taken over by adipose tissue (red arrows) and there was

Table 3
Antioxidant enzymes and malondialdehyde levels of alloxan-induced diabetic rats orally administered chromatographic fractions of *C. albidum* stem bark.

Treatment Group	SOD (unit/mg protein)	Catalase (unit/mg protein)	Malondialdehyde (nmol/L)
Control	4.924 ± 0.68 ^a	334.216 ± 37.02 ^a	2.062 ± 0.77 ^a
DR + 1 ml of DW	0.764 ± 0.19 ^b	123.183 ± 25.08 ^b	3.763 ± 0.48 ^b
DR + 2.5 mg/kg b. wt of glibenclamide	2.517 ± 0.36 ^c	194.885 ± 23.04 ^c	2.43 ± 0.55 ^a
DR + 2.5 mg/kg b. wt of F 5	2.761 ± 0.36 ^c	266.699 ± 41.22 ^d	1.96 ± 0.45 ^a
DR + 2.5 mg/kg b. wt of F7	4.395 ± 0.26 ^a	346.162 ± 82.08 ^a	2.03 ± 0.16 ^a

Values are means of ten replicates ± SEM.
Values with different superscripts are significantly different from each other (p < 0.05).

Where
Control = non-diabetic rats receiving 1 ml of distilled water; DC = diabetic rats receiving 1 ml of distilled water; RD = diabetic rats receiving standard drug; F5 = diabetic rats receiving chromatographic fraction 5; F7 = diabetic rats receiving chromatographic fraction 7

INSET (loose irregular connective tissues that are severely infiltrated (stars)).

4. Discussion

Hyperglycemia, the hallmark of diabetes, has been implicated in a number of pathways that contribute to cellular dysfunction and damage associated with micro- and macrovascular complications.²⁷ In this study, the significant upsurge in fasting blood glucose level of the diabetic control group might be due to obliteration of the insulin producing beta cells of the pancreas by alloxan. The resulting hyperglycaemia might be due to the summation of glucose fluxes from gluconeogenesis, glycogenolysis, lipogenesis and other pathways resulting from lack of insulin production.²⁸ Administration of F5 and F7 however, reduced the fasting blood glucose level of the alloxan-induced diabetic rats. This effect might be due to the phytochemicals (procyanidin B5, stigmasterol, catechin, epicatechin, epigallocatechin, eleagnine, eicosane, exadecane, octadecanoic acid) present in the plant which might be acting singly or in synergy with others and might be responsible for the restoration of the pancreas that resulted in hypoglycaemic effect of the chromatographic fractions observed in this study. This suggests that the fraction might be acting by restoring the pancreas histoarchitecture.

Oxidative stress is usually accompanied by an increased level of inflammation brought about by hyperglycemia in diabetics.²⁹ Glucose autooxidation and rearrangement to enediol radical ion³⁰ is capable of reducing molecular oxygen to superoxide anion that may oxidize lipids. Antioxidant agents impedes oxidative stress and protect against cellular damage by boosting the antioxidant enzyme capacity of the body.³¹

The decrease in the antioxidant enzyme system with increase in malondialdehyde level of the diabetic control group in this study

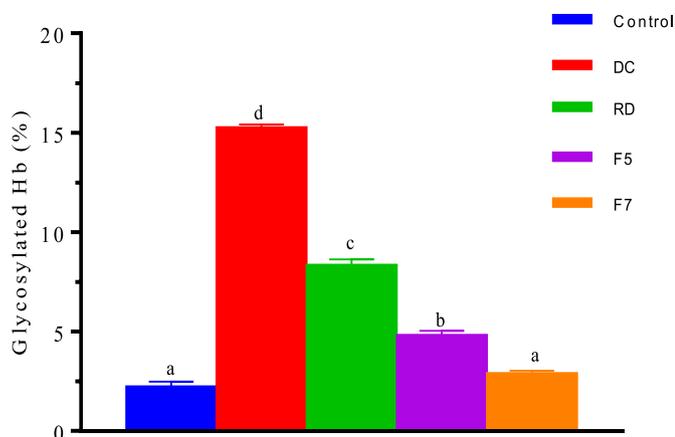


Fig. 2. Glycosylated haemoglobin of alloxan-induced diabetic rats orally administered chromatographic fractions of *Chrysophyllum albidum* stem bark for 14 days. Bars with different superscripts are significantly different from each other ($p < 0.05$).

Where:

Control = non-diabetic rats receiving 1 ml of distilled water; DC = diabetic rats receiving 1 ml of distilled water; RD = diabetic rats receiving standard drug; F5 = diabetic rats receiving chromatographic fraction 5; F7 = diabetic rats receiving chromatographic fraction 7

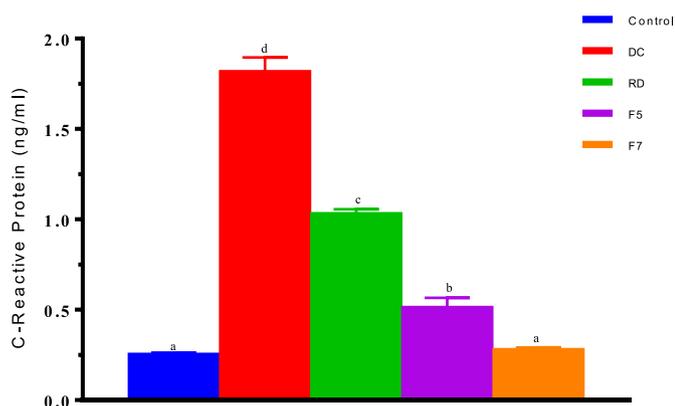


Fig. 3. C-reactive protein of alloxan-induced diabetic rats orally administered chromatographic fractions of *Chrysophyllum albidum* stem bark for 14 days.

Where:

Control = non-diabetic rats receiving 1 ml of distilled water; DC = diabetic rats receiving 1 ml of distilled water; RD = diabetic rats receiving standard drug; F5 = diabetic rats receiving chromatographic fraction 5; F7 = diabetic rats receiving chromatographic fraction 7

might be due to oxidative stress and lipid peroxidation correspondingly. Administration of the fractions of the extracts however boosted the antioxidant enzyme system and reduced the malondialdehyde level. This suggests that the fraction improved SOD

concentration to provide first line of defense against reactive oxygen species mediated cell injury by converting the proportion of superoxide anion to molecular oxygen and peroxide³² while catalase converts the peroxide formed into water and oxygen. The reduction in the MDA level observed in this study might be due to both impairment of glucose autoxidation potentiated by the phytochemicals present in the fractions of the extract and decrease generation of reactive oxygen species facilitated by increased antioxidant enzyme activity.³³

Persistent hyperglycaemia may lead to non-enzymic glycation of proteins which may induce cellular damage.³⁴ Glycosylated haemoglobin is a reliable index to monitor glucose lowering therapy and long-term glycaemia control.³⁵ In diabetic condition, blood glucose rejoins non-enzymatically with hemoglobin and accordingly increased glycosylated hemoglobin concentration.³⁶ The risk of developing microvascular complications rises exponentially with increasing glycosylated hemoglobin above the normal level (7.5)^{37,38} Every 1% increase in glycosylated hemoglobin is accompanied by 36% increase risk of microalbuminuria.³⁷ The risk of retinopathy also increases exponentially with increasing HbA1c and approximately 50% of those with a mean HbA1c of 10% or more will develop background retinopathy within 12 years from diagnosis of diabetes.³⁹

The substantial rise in the levels of glycosylated haemoglobin in the diabetic control group might be due to non-enzymatic glycosylation of the surplus glucose in circulation. The decrease in elevated glycosylated haemoglobin following oral administration of chromatographic fractions of the extract might be adduced to the ability of the extract to tightly control glycemic level. This suggests that the extract was able to prevent the autoxidation of glucose, the non-enzymatic and progressive glycation of protein while enhancing glucose flux into other pathways for effective glucose utilization.

C-reactive protein (CRP), a plasma protein made by the liver, is a sensitive and dynamic systemic indicator of inflammation.⁴⁰ The significant increase in the CRP level of the diabetic control group might be due to inflammation of the pancreas.⁴¹ This suggests a chronic hyperglycaemia which has been reported to increase the circulating inflammatory biomarkers.⁴² The significant decrease in CRP level of the extract treated diabetic rats suggests that the extract ameliorated inflammation and prevent secondary complications via reduction of CRP level. This finding agrees with the report of Mahluji et al.⁴³

Dyslipidemia, an abnormal metabolism of lipoproteins is central to poorly managed diabetes,⁴⁴ usually characterized by decrease HDL and increase total cholesterol, triglycerides, low density lipoprotein, very low density lipoprotein.⁴⁵ This disturbance in lipid profile is a major risk factor for coronary heart disease⁴⁶ and a marker of atherosclerosis. The significant rise in cholesterol, triglycerides, low density lipoprotein and very low-density lipoprotein and atherogenic index with decrease in HDL level in the

Table 4

Serum Lipids of Alloxan-induced Diabetic Rats Orally Administered Ethyl Acetate Chromatographic Fractions of *Chrysophyllum albidum* Stem Bark for 14 days.

Treatment Group	Cholesterol	Triglyceride	HDL-C	LDL-C	VLDL-C	Atherogenic index (LDL-C/HDL-C)
Control	26.12 ± 0.5 ^d	4.14 ± 0.5 ^a	16.69 ± 1.5 ^d	8.60 ± 1.6 ^c	0.83 ± 0.1 ^a	0.52 ± 0.1 ^b
DC	57.92 ± 1.0 ^e	26.98 ± 0.9 ^c	3.55 ± 0.6 ^a	48.98 ± 1.2 ^d	5.40 ± 0.2 ^c	14.12 ± 2.5 ^c
RD	11.04 ± 0.6 ^a	10.30 ± 0.3 ^b	7.28 ± 0.7 ^b	1.69 ± 0.2 ^a	2.06 ± 0.1 ^b	0.23 ± 0.0 ^a
F 5	15.65 ± 0.6 ^b	3.21 ± 0.45 ^a	11.41 ± 1.1 ^c	3.60 ± 0.55 ^b	0.64 ± 0.1 ^a	0.32 ± 0.1 ^a
F7	17.20 ± 0.8 ^c	3.95 ± 0.4 ^a	12.25 ± 1.3 ^c	4.16 ± 0.7 ^b	0.79 ± 0.1 ^a	0.35 ± 0.1 ^a

Values are means of ten replicates ± SEM.

Values with different superscripts are significantly different from each other ($p < 0.05$). Where: Control = non-diabetic rats receiving 1 ml of distilled water; DC = diabetic rats receiving 1 ml of distilled water; RD = diabetic rats receiving standard drug; F5 = diabetic rats receiving chromatographic fraction 5; F7 = diabetic rats receiving chromatographic fraction 7.

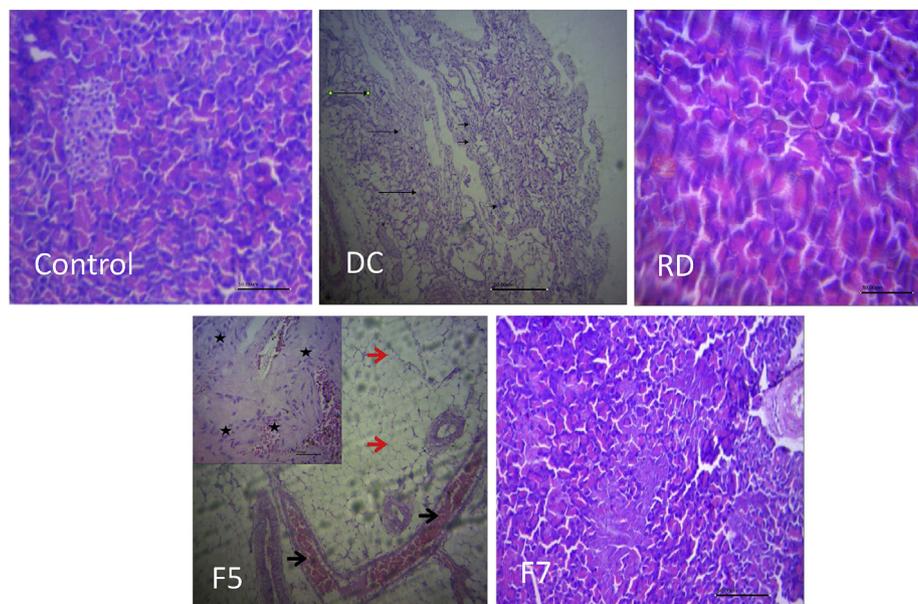


Plate 1. Cross section of rat pancreas administered fractions of *C. albidum* Stem Bark, Control: The pancreas appeared normal with no visible lesion seen, DC: The Parenchymatous portion of acini and islet portions completely obliterated (acini and islet not seen in the section), loose connective tissue and adipose tissue seen (long arrows) and they are highly infiltrated by inflammatory cells (short arrows); scale bar: 50 μm , RD: No visible lesion seen, F5: There is a severe congestion of the blood vessels (black arrows). The parenchyma is almost taken over by adipose tissue (red arrows). INSET: Loose irregular connective tissues are severely infiltrated (stars). (Mag: x100; inset scale bar: 5 μm), F7: The pancreas appeared normal with no visible lesion seen.

diabetic control group might be due to decreased insulin secretion which triggered the mobilization of free fatty acid from the fat deposit. Administration of the chromatographic fractions (F5 and F7) however significantly decreased cholesterol, triglycerides, low density lipoprotein and very low-density lipoprotein, atherogenic index and increased HDL level. This might be due to brisk release of insulin from the pancreas activating lipoprotein lipase which hydrolyses triglycerides and inhibits the release of free fatty acids from the fat stores.⁴⁷ This suggests that the chromatographic fractions of the extract might be preventing dyslipidemia by stimulating fatty acid biosynthesis, increase utilization of glucose and decrease mobilization of free fatty acids from the stores and incorporation of free fatty acids into triglycerides in the liver and adipose tissues.

5. Conclusion

Based on the results obtained from this study, it could be concluded that 2.5 mg/kg body weight of F5 and F7 reduces fasting blood glucose, boost antioxidant enzyme activity and ameliorate some markers of diabetes complications. However, F7 demonstrated the most profound effects and restored the distorted pancreas histoarchitecture and can therefore be explored for the management of diabetes.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

References

- Yaribeygi H, Atkin SL, Sahebkar A. A review of the molecular mechanisms of hyperglycemia-induced free radical generation leading to oxidative stress. *J Cell Physiol.* 2019;234(2):1300–1312. <https://doi.org/10.1002/jcp.27164>.
- Hunt JV, Dean RT, Wolff SP. Hydroxyl radical production and autoxidative glycosylation. Glucose autooxidation as the cause of protein damage in the experimental glycation model of diabetes mellitus and ageing. *Biochem J.* 1988;256(1):205–212. <https://doi.org/10.1042/bj2560205>.
- Pickering RJ, Rosado CJ, Sharma A, Buksh S, Tate M, de Haan JB. Recent novel approaches to limit oxidative stress and inflammation in diabetic complications. *Clin Transl Immunol.* 2018;7(4):1–20. <https://doi.org/10.1002/cti2.1016>.
- Newsholme P, Cruzat V, Arfuso F, Keane K. *Nutrient Regulation of Insulin Secretion and Action.* 2014. <https://doi.org/10.1530/JOE-13-0616>. Published online.
- Onyeka CA, Aligwekwe AU, Olawuyi TS, Nwakanma AA, Kalu ECOA. Antifertility effects of ethanolic root bark extract of *Chrysophyllum albidum* in male albino rats. *Int J Appl Res Nat Prod.* 2012;5(1):12–17.
- Adebayo AH, Abolaji AO, Opata TK, Adegbenro IK. Effects of ethanolic leaf extract of *Chrysophyllum albidum* G. on biochemical and haematological parameters of albino Wistar rats. 2010;9(14):2145–2150.
- Okoli BJ, Okere OS. Antimicrobial activity of the phytochemical constituents of *Chrysophyllum albidum* G. Don. (African star apple) plant. *J Res Natl Dev.* 2010;8(1):301–311.
- Yusuf BO, Yakubu MT, Akanji MA. Anti-diabetic activity of *Chrysophyllum albidum* (G. Don) stem bark in alloxan-induced type 1 diabetic female wistar rats. *Tanzan J Sci.* 2020;6(3).
- Idowu TO, Ogundaini AO, Adesanya SA, et al. Afr J Tradit Complement Altern Med. (2016) 13(5): 182–189 ANTIBACTERIAL PROPERTIES Idowu et al. *Afr J Tradit, Complementary Altern Med.* 2016;13(5):182–189. <https://doi.org/10.21010/ajtcam.v13i5.24>, 2016;13:182–189.
- Yakubu MT, Akanji MA, Oladiji AT. Aphrodisiac potentials of the aqueous extract of *Fadogia agrestis* (Schweinf. Ex Hiern) stem in male albino rats. *Asian J Androl.* 2005;7(4):399–404. <https://doi.org/10.1111/j.1745-7262.2005.00052.x>.
- Muhit MA, Tareq SM, Apu AS. BD and IM. Isolation and identification of compounds from the leaf extract of *Dillenia indica* Linn. *Bangladesh Pharm J.* 2010;13(1):49–53.
- Sharma AK, Bharti S, Goyal S, et al. Upregulation of PPAR γ by Aegle marmelos ameliorates insulin resistance and β -cell dysfunction in high fat diet fed streptozotocin induced type 2 diabetic rats. *Phyther Res.* 2011;25(10):1457–1465. <https://doi.org/10.1002/ptr.3442>.
- Yakubu MT, Ogunro OB. Effects of aqueous extract of *Fadogia agrestis* stem in alloxan-induced diabetic rats. *Bangladesh J Pharmacol.* 2014;9(3):356–363. <https://doi.org/10.3329/bjp.v9i3.18610>.
- Oloyede HOB, Bello TO, Ajiboye TO, Salawu MO. Antidiabetic and anti-dyslipidemic activities of aqueous leaf extract of *Dioscoreophyllum cumminsii* (Stapf) Diels in alloxan-induced diabetic rats. *J Ethnopharmacol.* 2015;166:313–322. <https://doi.org/10.1016/j.jep.2015.02.049>.
- Misra HP, Fridovich I. The role of superoxide dismutase anion in the auto oxidation of epinephrine and a simple assay for superoxide dismutase. *J Biochem.* 1972;247:3170–3175.
- Beers RF, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol Chem.* 1952;195(1):133–140.
- Varshney R, Kale RK. Effects of Calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. *Int J Radiat Biol.* 1990;58:733–743.

20. Trivelli LA, Ranney HM, Lai H-T. Hemoglobin components in patients with diabetes mellitus. *N Engl J Med.* 1971;284(7):353–357.
21. Burtis CA, Ashwood ERDEB. In: *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics.* fifth ed. Elsevier Saunders; 1999.
22. Roeschlau P, Bernst E, Gruber JW. No Title. *Biochem Clin Chem Clin.* 1974;(2):403.
23. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann Clin Biochem.* 1969;6(1):24–27.
24. Lopes-Virella MF, Stone P, Ellis S, JAC. Cholesterol determination in high-density lipoproteins separated by three different methods. *Clin Chem.* 1977;23(5):882–884. <https://doi.org/10.1093/clinchem/23.5.882>.
25. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* 1972;18(6):499–502.
26. Drury RAB, Wallington EA. In: *Carleton's Histological Techniques.* fourth ed. Oxford University Press; 1980.
27. King GL. The role of inflammatory cytokines in diabetes and its complications. *J Periodontol.* 2008;79(8s):1527–1534. <https://doi.org/10.1902/jop.2008.080246>.
28. Petersen MC, Vatner DF, Shulman GI. Regulation of hepatic glucose metabolism in health and disease. *Nat Rev Endocrinol.* 2017;13(10):572–587. <https://doi.org/10.1038/nrendo.2017.80>.
29. A AK. Anzer honey prevents N-ethylmaleimide-induced liver damage in rats. 2009;61:333–337. <https://doi.org/10.1016/j.etp.2008.07.005>.
30. Wolff SP, Dean RT. Glucose autooxidation and protein modification. The potential role of "autooxidative glycosylation" in diabetes. *Biochem J.* 1987;245(1):243–250. <https://doi.org/10.1042/bj2450243>.
31. Mohamed M, Sulaiman SA, Jaafar H, Sirajudeen KNS. Effect of different doses of Malaysian honey on reproductive parameters in adult male rats. *Published online.* 2012:182–186. <https://doi.org/10.1111/j.1439-0272.2010.01159.x>.
32. Tiwari BK, Pandey KB, Abidi AB, Rizvi SI. *Markers of Oxidative Stress during Diabetes Mellitus.* vol. 2013. 2013.
33. Ullah A. Diabetes mellitus and oxidative stress — A concise review. *Saudi Pharmaceut J.* 2016;24(5):547–553. <https://doi.org/10.1016/j.jps.2015.03.013>.
34. Tomlinson DR, Gardiner NJ. *Glucose neurotoxicity.* 2008;9(january). <https://doi.org/10.1038/nrn2294>.
35. Sasikala C, Sudhakar Y. Effect of compounds isolated from *filicium decipiens* and *ventilago madraspatana* against diabetic nephropathy in streptozotocin induced diabetic rats. *Indian J Pharm Educ Res.* 2015;49(2):146–151. <https://doi.org/10.5530/ijper.49.2.11>.
36. Schmidt AM, Editor G, Giacco F, Brownlee M. Oxidative stress and diabetic complications IR RAGE SOD. *Published online.* 2010:842–853. <https://doi.org/10.1161/CIRCRESAHA.110.223545>.
37. Schultz CJ, Konopelska-Bahu T, Dalton RN, et al. Microalbuminuria prevalence varies with age, sex, and puberty in children with type 1 diabetes followed from diagnosis in a longitudinal study. *Diabetes Care.* 1999;22(3):495–502. <https://doi.org/10.2337/diacare.22.3.495>.
38. Weber B, Burger W, Hartmann R, Hovener GMR, OU. Risk factors for the development of retinopathy in children and adolescents with Type 1 (insulin-dependent) diabetes mellitus. *Diabetologia.* 1986;29:23–29.
39. Danne T, Weber B, Hartmann R, Enders I, Burger W, Hovener G. Long-term glycemic control has a nonlinear association to the frequency of background retinopathy in adolescents with diabetes: follow-up of the Berlin retinopathy study. *Diabetes Care.* 1994;17(12):1390–1396. <https://doi.org/10.2337/diacare.17.12.1390>.
40. Pepys MB, Hirschfield GM. C-reactive protein : a critical update. 2003;111(12):1805–1812. <https://doi.org/10.1172/JCI200318921.Introduction>.
41. Mirochnik R, Araida S, Yaffe V, El-naaj IA. C-reactive protein concentration as a prognostic factor for inflammation in the management of odontogenic infections. *Br J Oral Maxillofac Surg.* 2017:1–5. <https://doi.org/10.1016/j.bjoms.2017.10.006>. Published online.
42. Spranger J, Kroke A, Mo M, Hoffmann K, Bergmann MM. Inflammatory cytokines and the risk to develop type 2 diabetes. *Diabetes.* 2003;52(March):812–817.
43. Mahluji S, Ostadrahimi A, Mobasser M, Attari VE, Payahoo L. Anti-inflammatory effects of zingiber officinale in type 2 diabetic patients. 2013;3(2):273–276.
44. Jacobson TA, Miller M, Schaefer E. *Hypertriglyceridemia and Cardiovascular Risk Reduction.* 2007;29(5):763–777.
45. Lee J, Lee HI, Seo K II, et al. Effects of ursolic acid on glucose metabolism, the polyol pathway and dyslipidemia in non-obese type 2 diabetic mice. *Indian J Exp Biol.* 2014;52(7):683–691.
46. Bhutto MG, Lokesh MR, Sharath KDS, Afroze MKH, Ghouse PAD. Association between lipid profile and silent coronary artery disease in south Indian patients with type 2 diabetes mellitus. *Int J Adv Med.* 2017;(May). <https://doi.org/10.18203/2349-3933.ijam20164020>.
47. Kehlenbrink S, Koppaka S, Martin M, Relwani R. Elevated NEFA levels impair glucose effectiveness by increasing net hepatic glycogenolysis. *Published online.* 2012:3021–3028. <https://doi.org/10.1007/s00125-012-2662-6>.