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Original Research Article

Antioxidant and Hypoglycaemic Effects of *Ardisia Compressa* (HBEK, Myrsinaceae) Extract in Type 2 Diabetic Rats

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Abstract

Purpose: *To evaluate the possible hypoglycaemic, hypolipidemic, and antioxidant activities of Ardisia compressa (AC) on a rat model of type 2 diabetes.*
Methods: *Diabetes was induced in female Wistar rats by intraperitoneal (i.p.) administration of streptozotocin (65 mg/kg) and nicotinamide (120 mg/kg). The diabetic animals were orally administered water with or without metformin 150 mg/kg (D+Met) or 100, 200, or 400 mg/kg AC (D+100, 200 or 400), daily for 21 days. Normoglycaemic animals were given water with or without 400 mg/kg AC. Glycaemia, urinary protein excretion, lipid profiles, and antioxidant activity were determined.*
Results: *AC decreased hyperglycaemia in diabetic animals (150.67 ± 13.41 mg/dL, AC vs. 346.33 ± 51.21 mg/dL, Diabetes), but not hyperlipidemia. An antioxidant effect was also observed in the 400-mg/kg AC extract group, which exhibited significantly decreased lipid peroxidation (2.597 ± 0.284, AC vs. 3.623 ± 0.280 µM malondialdehyde [MDA]/g, Diabetes) and reactive oxygen species (ROS) production (1.533 ± 0.207, AC vs. 5.281 ± 0.457 µg DCF/mg, Diabetes) in liver. In addition, lipid peroxidation, ROS, and oxidised proteins levels were decreased in the kidneys and pancreas of AC treated diabetic animals.*
Conclusion: *AC leaves exert hypoglycaemic and antioxidant effects in type 2 diabetic rats, and has the potential to delay or prevent the onset of diabetes-induced complications.*

Keywords: *Type 2 diabetes, Ardisia compressa, Hypoglycaemia, Lipidaemia, Reactive oxygen species, Oxidative stress*

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INTRODUCTION

Modern lifestyle changes have led to increases in overweight and obesity prevalence rates among populations; therefore, there is an associated rise in the incidence of diabetes that is currently considered a worldwide health problem. The World Health Organization (WHO) estimates that there are more than 347 million people with diabetes worldwide, and type 2 diabetes (T2D) is the most common form [1].

It has been reported that in the diabetic state, increases in the oxidative damage of lipids, DNA, and proteins are due to greater levels of reactive oxygen species (ROS), which leads to cellular injury related to the onset of many diabetic complications [2]. The first line of treatment is

lifestyle modification; oral hypoglycaemic drugs are indicated when diet and exercise are not able to achieve acceptable glycaemic control [3].

Pharmacologic therapy has disadvantages, and patients can exhibit resistance or decreased treatment response or experience adverse effects. Thus, herbal alternatives are being explored for their ability to provide symptomatic relief prevent diabetic complications [3]. It is estimated that Mexico has approximately 306 native species of plants with hypoglycaemic activity that belong to 235 genera and 93 families [4]; one of these potential T2D treatments is *Ardisia compressa* (chagalapoli). Different metabolites of pharmacological interest have been isolated from several *Ardisia* species [5]. Specifically, *Ardisia compressa* (AC) has been shown to exert different biological activities, such as anticarcinogenicity on human colon cells [5] and rat liver [6], and anticytotoxic and antigenotoxic effects on rat hepatocytes exposed to benomyl and 1-nitropyrene [7,8].

These biological activities are thought to be due to AC's antioxidant properties. Because the hypoglycaemic activity of AC has not been proven scientifically and diabetes and its complications are related to oxidative stress, the purpose of the present work was to investigate the possible hypoglycaemic, hypolipidemic, and antioxidant effects of AC on T2D rats.

EXPERIMENTAL

Plant material

AC leaves were obtained from Sontecomoapan, Los Tuxtlas Veracruz, Mexico in August 2011. The plant material was authenticated by Biol. Laura Doval-Ugalde of the National School of Biological Sciences (ENCB), IPN, Ciudad de Mexico, Mexico. A voucher specimen (no. 85033) was deposited in the Herbarium of National School of Biological Sciences, IPN.

Phytochemical screening

Aqueous and ethanol extracts were prepared for phytochemical screening to identify several constituents by colorimetric and gravimetric methods using reagents or reactions described elsewhere [9].

Extract preparation

The aqueous extract was prepared by adding 50 g finely ground AC leaves to 1000 mL boiling distilled water. The aqueous extract was then filtered, freeze-dried, and stored at -15 °C.

Animals

Adult female Wistar rats weighing 220 ± 20 g obtained from the Animal House of the Autonomous University of State of Hidalgo were conditioned for 7 days in polypropylene cages, under light-dark cycles of 12 h and fed with standard feed and water *ad libitum*. The experimental protocols were approved by the Bioethical Committee of the National School of Biological Sciences of National Polytechnic Institute. International guidelines [10] and the Mexican Official Standard (NOM-062-ZOO-1999) concerning technical specifications for production, care, and use of laboratory animals were followed.

Induction of T2D

T2D was induced by a single intraperitoneal injection of 65 mg/kg body weight (b.w.) streptozotocin (STZ) (Sigma Chem. Co., St. Louis Missouri, USA) dissolved in 0.1 M citrate buffer (pH 4.5) followed by intraperitoneal (i.p.) injection of 120 mg/kg b.w. nicotinamide (NA) dissolved in isotonic saline solution [11]. On day seven, after a 6-h fasting period, diabetic state was confirmed by measuring glucose levels on a blood sample obtained from the caudal vein (glucometer Accu-Chek Performa, Roche Diagnostics). Rats with blood glucose levels greater than 130 mg/dL were considered as diabetic.

AC's effect on hypoglycaemia

Animals were randomised into seven groups of six rats. The first and second groups were comprised of normoglycaemic animals that received water and 400 mg/kg b.w. lyophilised AC extract, respectively. The other five groups were diabetic rats: untreated, metformin (150 mg/kg b.w.) or the lyophilised AC extract (100, 200, and 400 mg/kg b.w.). All treatments were orally administered daily for 21 days. Each week, b.w. and blood glucose were measured.

Oral glucose tolerance test

Basal blood glucose was measured on day 18, after a 6 h fasting period. The respective treatment was then administered, and 30 minutes later, animals received an oral glucose load of 2 g/kg b.w. Glycaemic values were measured on tail vein by using a glucometer Accu-Chek Performa (Roche Diagnostics) at 0, 30, 60, and 120 min after glucose administration.

Biochemical assays

Lipid profiles including total cholesterol, triglycerides and high-density lipoprotein (HDL) were determined at 21 days with a Wiener Laboratory Selectra 2 automatic analyser (Spankeren, the Netherlands). Rat urine was collected by using metabolic cages for 5-6 h. Urine protein concentrations were measured according to the Bradford method [12]; urinary flow and urinary protein excretion were also calculated.

At the end of the experiment, the animals were sacrificed by decapitation following the administration of ethyl ether anaesthesia. Liver, kidney, and pancreas samples were immediately obtained, and each tissue was homogenised individually with phosphate-buffered saline (PBS). Oxidative stress biomarkers were determined as follows: ROS were measured with a sensitive fluorescent probe DCFH-DA (2´,7´-dichlorodihydrofluorescein diacetate) [13], lipid peroxidation was quantified by measuring thiobarbituric acid reactive substances (TBARS) [14], and oxidised proteins were assessed their formation of complexes with DNPH (2,4-dinitrophenylhydrazine) [15].

Statistical analysis

Data are expressed as the mean ± SEM (standard error of the mean). Results were analysed for statistical significance by one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls method using SigmaPlot ver. 11 software, with $p < 0.05$ considered significant.

RESULTS

Phytochemical profile of plant extract

Aqueous AC extract was found to contain reducing sugars, coumarins, triterpenoid saponins, and tannins (polyphenolic compounds and gallic acid derivatives).

Hypoglycaemic effect of AC extract

Over the course of 21 days, the blood glucose levels of rats treated with metformin or 100, 200, and 400 mg/kg b.w. AC extract were significantly and gradually decreased (Table 1) with respect to the diabetic control group, but they did not achieve the blood glucose levels of the normoglycaemic groups.

Oral glucose tolerance

In the oral glucose tolerance test, a significant difference was only found between the normoglycaemic and diabetic groups (Table 2), but there were no differences among the treated groups.

Table 1: Hypoglycaemic effect of lyophilized aqueous AC extract

Group	Blood glucose (mg/dL)			
	0 day	7th day	14th day	21th day
N	89.17 ± 2.82	87.00 ± 4.99	90.33 ± 3.94	90.83 ± 4.47
N+400	84.00 ± 3.33[b]	87.83 ± 3.85[b]	82.00 ± 3.88[b]	85.17 ± 2.93[b]
D	376.00 ± 38.06[a]	367.33 ± 44.87[a]	393.17 ± 52.32[a]	346.33 ± 51.21[a]
D+MET	262.50 ± 33.16[a]	187.33 ± 39.32[b]	152.33 ± 22.74[a,b]	193.83 ± 41.01[a,b]
D+100	209.50 ± 34.29[a,b]	213.50 ± 57.32[a]	204.83 ± 56.98[a,b]	168.17 ± 29.61[a,b]
D+200	241.67 ± 40.90[a,b]	298.83 ± 34.43[a]	215.33 ± 51.69[a,b]	208.33 ± 45.70[a,b]
D+400	216.17 ± 38.12[a,b]	234.17 ± 52.79[a]	154.83 ± 34.61[a,b]	150.67 ± 13.41[a,b]

N = normal control; N+400 = N given 400 mg/kg AC extract; D = Diabetic control; D+MET = D given 150 mg/kg metformin; D+100, D+200, and D+400 = D given 100, 200 or 400 mg/kg AC extract. [a] p<0.05 vs. N; [b] p < 0.05 vs. D; n = 6, mean ± SEM

Table 2: Oral glucose tolerance of rats

Group	Blood glucose (mg/dL)			
	0 min	30 min	60 min	120 min
N	88.50 ± 2.11	122.00 ± 6.44	92.17 ± 5.49	79.00 ± 3.67
N+400	85.50 ± 4.29	110.50 ± 3.42	107.67 ± 5.12	94.17 ± 6.80
D	299.50 ± 63.45[a]	396.50 ± 68.78[a]	380.00 ± 56.95[a]	347.50 ± 34.61[a]
D+MET	209.17 ± 46.93[a]	214.67 ± 50.69[a,b]	203.33 ± 45.35[a,b]	137.50 ± 25.67[a,b]
D+100	192.66 ± 48.85[a]	256.17 ± 53.79[a,b]	280.00 ± 68.34[a]	202.17 ± 55.71[a]
D+200	281.50 ± 51.87[a]	398.83 ± 56.57[a]	387.33 ± 50.39[a]	292.00 ± 44.25[a]
D+400	209.67 ± 42.30[a]	250.67 ± 48.28[a,b]	279.17 ± 57.72[a]	235.33 ± 48.81[a]

N = normal control; N+400 = N given 400 mg/kg AC extract; D = Diabetic control; D+MET = D given 150 mg/kg metformin; D+100, D+200, and D+400= D given 100, 200, and 400 mg/kg AC extract. . [a] p < 0.05 vs. N; [b] p < 0.05 vs. D; *n = 6, mean ± SEM*

Biochemical assays

Lipid profiles were not affected by diabetes; metformin; or AC extract at 100, 200, or 400 mg/kg b.w. (data not shown). Urinary protein excretion (Fig 1) in diabetic controls was significantly increased compared to the normoglycaemic group (0.308 ± 0.02 and 0.035 ± 0.01 µg/min, respectively).

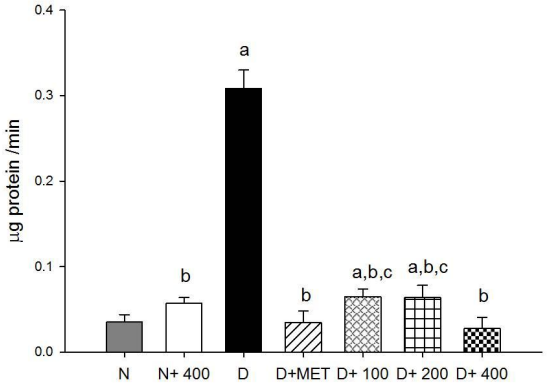

Fig 1: Urinary protein excretion of diabetic rats treated with lyophilized AC extract for 21 days (n = 6, mean ± SEM). N = normal control; N+400 = N given 400 mg/kg AC extract; D= Diabetic control; D+MET = D given 150 mg/kg metformin; D+100, D+200, and D+400 = D given 100, 200, and 400 mg/kg AC extract; [a] $p < 0.05$ vs. N, [b] $p < 0.05$ vs. D, and [c] $p < 0.05$ vs. D+MET

Metformin (0.035 ± 0.01 µg/min) or AC extract (100, 200, and 400 mg/kg b.w.) produced a significant decrease in urinary protein excretion with respect to that of the diabetic control group, and treatment with AC at 400 mg/kg (0.028 ± 0.013 µg/min) resulted in values closest to those of the normoglycaemic and metformin groups.

Oxidative stress

Liver lipid peroxidation (Fig 2A) was significantly increased in diabetic controls (3.62 ± 0.28 µM MDA/g) compared to that of the normal control group (2.26 ± 0.26 µM MDA/g). Administration of 400 mg/kg AC extract significantly decreased MDA levels (2.60 ± 0.28 µM MDA/g) with respect to the diabetic control group, reaching values similar to normoglycaemic animals.

ROS levels in the liver (Fig 2B) were significantly increased in the diabetic group (5.28 ± 0.46 µg DCF/mg) with respect to normal control animals (2.32 ± 0.17 µg DCF/mg); however, this biomarker was significantly diminished in the groups treated with metformin and 100, 200, or 400 mg/kg b.w. AC (2.95 ± 0.49, 2.38 ± 0.21, 2.01 ± 0.27, and 1.53 ± 0.21 µg DCF/mg, respectively) in a dose-dependent manner. The highest decrease was achieved with the highest dose, values in the 400 mg/kg b.w. group were below those of the normoglycaemic group, and a significant decrease was observed from D+400 with respect to D+100. Protein carbonyls (CO) levels were not affected, and there were no significant differences among groups. (data not shown).

Lipid peroxidation levels in the kidneys (Fig 3A) of diabetic control animals were significantly elevated with respect to normal controls (1183.50 ± 25.33 and 329.50 ± 43.85 µM MDA/g, respectively). The administration of metformin (362.78 ± 59.54 µM MDA/g) and lyophilized AC

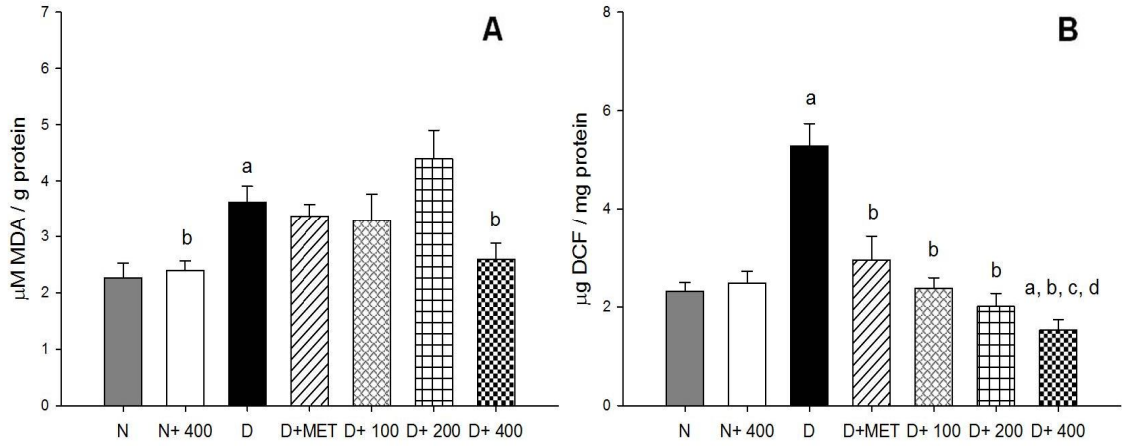

Fig 2: Levels of A) lipoperoxidation and B) ROS in the liver of normoglycaemic and diabetic rats treated with lyophilized AC extract; (n = 6, error bars denote SEM). N= normal control; N+400 = N given 400 mg/kg AC extract; D = Diabetic control; D+MET = D given 150 mg/kg metformin; D+100, D+200, and D+400 = D given 100, 200, and 400 mg/kg AC extract. [a] $p < 0.05$ vs. N, [b] $p < 0.05$ vs. D, [c] $p < 0.05$ vs. D+MET, and [d] $p < 0.05$ vs. D+100

extract at doses of 100, 200, and 400 mg/kg b.w. (890.51 ± 21.50, 768.16 ± 37.16, and 456.70 ± 27.39 µM MDA/g, respectively) significantly diminished (p<0.05) lipid peroxidation in the kidney compared to diabetic control; however, AC treatment was not as effective as metformin. Levels of oxidized proteins (Fig 3B) were also increased in the diabetic control group with respect to normal control (873.32 ± 30.33 and 367.47 ± 20.39 µM CO/g, respectively); metformin and 200 or 400 mg/kg b.w. lyophilized AC extract (469.21 ± 26.67, 748.85 ± 15.87, and 462.78 ± 27.24 µM CO/g, respectively) decreased protein carbonyl levels compared to diabetic group. The values between the group treated with the highest dose of AC extract and those that received metformin were not significantly different. Kidney ROS levels (Fig 3C) in the diabetic control group were significantly higher than in normal control (4.74 ± 0.31 and 2.80 ± 0.33 µg DCF/mg, respectively). ROS values were significantly decreased by metformin and lyophilized AC extract administered at doses of 200 and 400 mg/kg (3.76 ± 0.14, 3.27 ± 0.32, and 2.78 ± 0.24 µg DCF/mg, respectively); the highest AC dose was able to reduce levels even more than the reference drug, and treatment with 400 mg/kg b.w. achieved values similar to those of the normoglycaemic group.

On the other hand, lipid peroxidation in the pancreas tissue (Fig 4A) of the diabetic control group was significantly increased with respect to the normal control group (14.48 ± 0.96 and 3.69 ± 0.52 µM MDA/g, respectively). Administration of metformin and different doses of AC extract significantly decreased lipid peroxidation levels (4.08 ± 0.45, 7.07 ± 0.47, 7.44 ± 0.65, and 5.83 ± 0.38 µM MDA/g, respectively) with respect to diabetic control; however, the groups treated with AC extract did not reach the low values measured in the metformin and normal control groups. Oxidized protein levels in the pancreas (Fig 4B) of diabetic controls were higher than those in the normal control group (861.72 ± 31.35 and 411.91 ± 18.13 µM CO/g, respectively); it was only reduced by administering metformin or 400 mg/kg b.w. AC extract (621.44 ± 19.60 and 631.42 ± 25.21 µM CO/g, respectively) with respect to diabetic control, but values observed in the normoglycaemic group were not achieved with either treatment. Fig. 4C shows that ROS levels in the pancreas of diabetic control animals was higher than in normal control (4.70 ± 0.38 and 1.44 ± 0.25 µg DCF/mg, respectively). Similar to

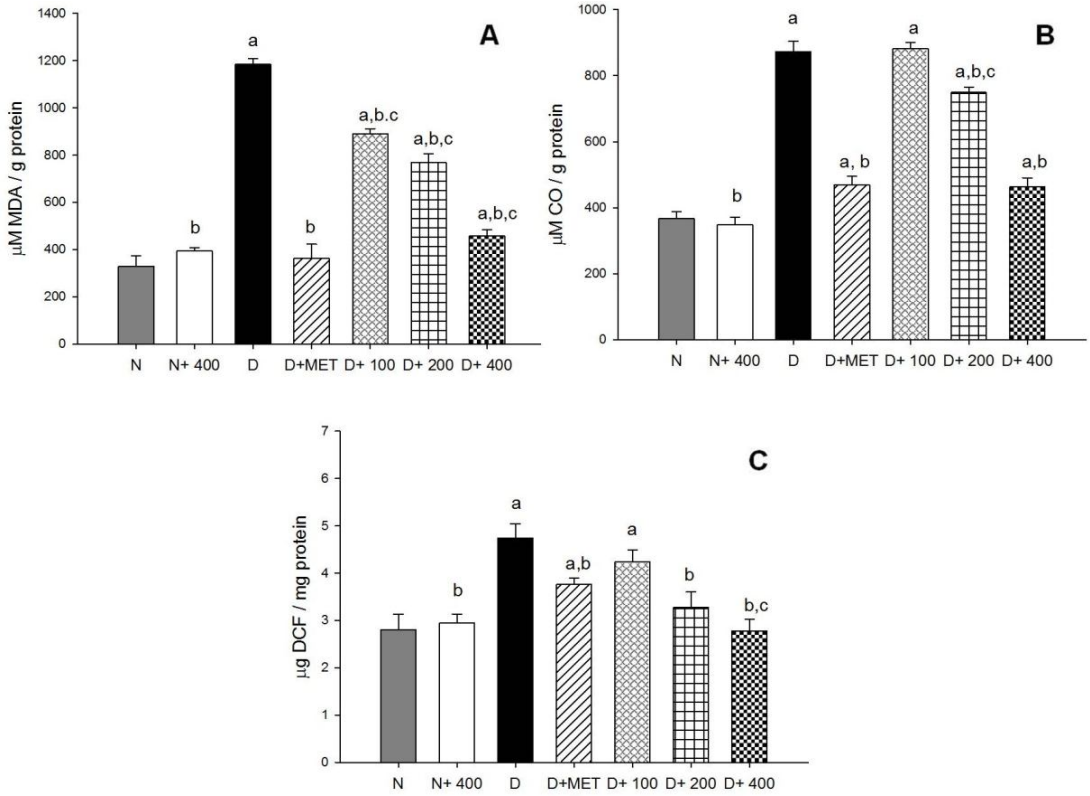

Fig 3: Levels of A) lipid peroxidation, B) protein carbonyls, and C) ROS in the kidney of diabetic rats treated with lyophilized AC extract for 21 days; (n=6, error bars SEM). N = normal control; N+400 = N given 400 mg/kg AC extract; D = Diabetic control; D+MET= D given 150 mg/kg metformin; D+100, D+200, and D+400= D given 100, 200, and 400 mg/kg AC extract; [a]p < 0.05 vs. N; [b] p < 0.05 vs. D, and [c]p < 0.05 vs. D+MET.

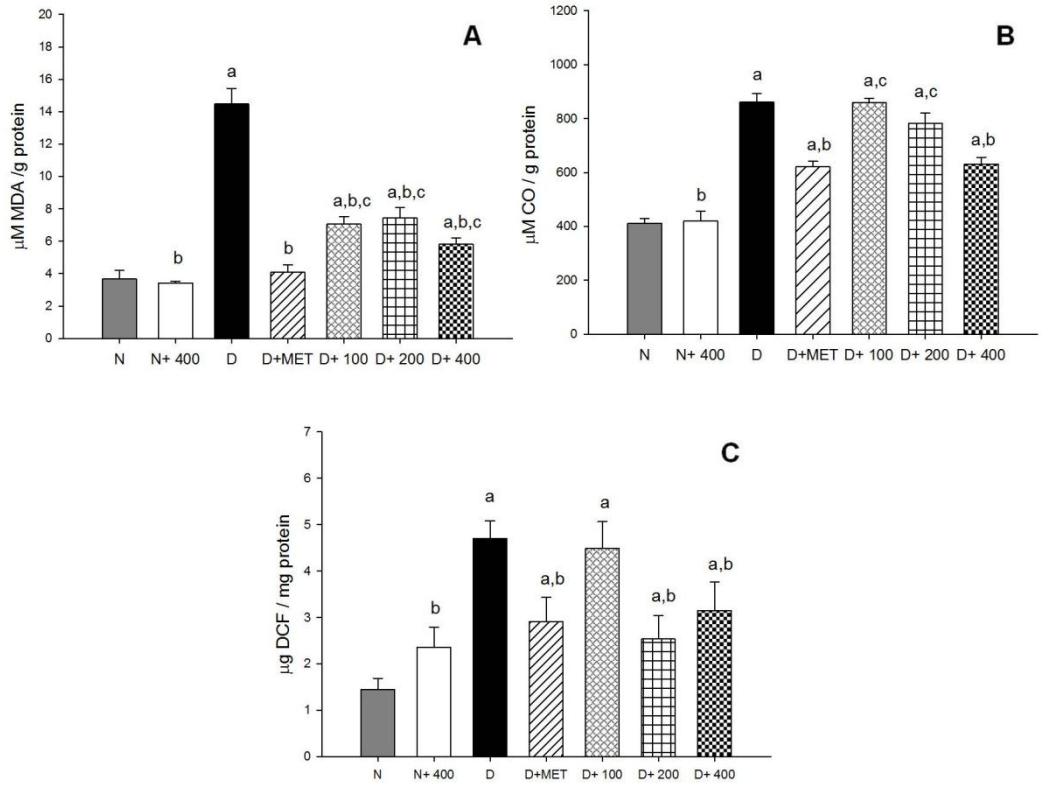

Fig 4: Levels of A) lipoperoxidation, B) protein carbonyls, and C) ROS in the pancreas of diabetic rats treated with lyophilized AC extract; (n = 6, error bars denote SEM). N = normal control; N+400 = N given 400 mg/kg AC extract; D= Diabetic control; D+MET= D given 150 mg/kg metformin; D+100, D+200, and D+400 = D given 100, 200, and 400 mg/kg AC extract; [a]$p < 0.05$ vs. N; [b]$p < 0.05$ vs. D; and [c]$p < 0.05$ vs. D+MET

the other results, administration of metformin or 200 or 400 mg/kg lyophilized AC extract (2.91 ± 0.53, 2.53 ± 0.51, and 3.15 ± 0.61 µg DCF/mg, respectively) decreased ROS levels with respect to the diabetic control group.

DISCUSSION

Medicinal plants have begun to gain scientific importance as a source of hypoglycaemic agents because they are rich in phenolic compounds, flavonoids, terpenoids, coumarins, and other constituents capable of reducing blood glucose levels [3]. AC has been used in traditional medicine in Veracruz, Mexico for the treatment of T2D; the present results demonstrate that AC contains reducing sugars, triterpenoid saponins, coumarins, and tannins (polyphenolic compounds and gallic acid derivatives). Moreover, we found the plant extract exerted a good hypoglycaemic effect in diabetic rats, possibly due to the presence of metabolites identified in the phytochemical screening.

Oxidative stress is an important factor in the development and progression of diabetes and subsequent complications that contribute to morbidity and mortality [2]. Hyperglycaemia increases oxidative stress, and free radicals might react with organic compounds to cause lipid peroxidation, fragmentation of proteins, and deamination of guanine and adenine in DNA, ultimately leading to gene mutation and cell damage [2]. Antioxidative compounds have been shown to demonstrate several beneficial effects in the prevention and treatment of diabetic complications. The kidney, liver, and pancreas are affected during a diabetic state [16,17], so we measured several oxidative stress biomarkers to determine if AC extract protected these organs.

Hyperglycaemia can lead to complications though five mechanisms: (1) the formation and activity of advanced glycated end products; (2) increased polyol pathway activity; (3) greater hexosamine pathway activity; (4) enhanced activity of protein kinase C; and (5) elevated oxidative stress [18] through glucose autoxidation or glycoxidation-forming intermediate oxidants, such as superoxide anions, hydroxyl radicals, and hydrogen peroxide, which may damage lipids, proteins, and DNA [19].

Polyphenolic compounds have important antioxidant activities, and they can help to prevent or avoid the progression of several

diseases and associated complications, including diabetes [16]. We found that lyophilized AC extract administered to diabetic rats at different doses decreased oxidative stress levels in the liver, kidney, and pancreas, with the greatest effects achieved at the highest dose.

The combination of oxidative stress and hyperglycaemia may play an important role in the pathogenesis of glomerular and tubular dysfunction and structural kidney abnormalities. Persistent hyperglycaemia-induced ROS production has been implicated in early glomerular podocyte injury, which contributes to early proteinuria, and experimental studies suggest that decreasing oxidative stress burden may prevent glomerular damage in diabetic nephropathy [20]. The decreases in urinary protein excretion and urinary flow in AC-treated groups may be explained by decreased ROS levels in the kidney, which could prevent or reduce the damage to podocytes and other kidney cells.

AC extract decreased lipid peroxidation and ROS levels in diabetic rat liver; however, oxidized protein levels were not affected by diabetes or by the different treatments, which may be due to organ-specific differences. Because the liver is the focal organ for oxidative and detoxifying processes, its antioxidant system is more efficient than those of other organs [16].

It has been reported that the pancreas is very susceptible to oxidative damage due to its low antioxidant levels making it more susceptible to glucotoxicity [17]. Lipid peroxidation was significantly diminished in the kidney and pancreas of animals treated with all doses of AC (100, 200, and 400 mg/kg). Oxidized proteins and ROS levels were also decreased, but only at doses of 200 and 400 mg/kg AC.

The findings described in the present study indicate that AC can attenuate damage to the liver, kidney, and pancreas caused by hyperglycaemia-generated oxidative stress.

CONCLUSION

The lyophilized aqueous extract of AC leaves exerts both hypoglycaemic activity and antioxidant effects on the liver, kidney, and pancreas of type 2 diabetic rats. Although more research is needed to explore other desirable effects, our results indicate that AC has the potential to delay or to prevent the onset of diabetes-induced complications.

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