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Antidiabetic and antihyperlipidemic effects of *Argyreia pierreana* and *Matelea denticulata*: Higher activity of the micellar nanoformulation over the crude extract

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ABSTRACT

Background and aim: Herbal medicine combined with nanotechnology is widely proposed to improve the oral bioavailability, reduce the required dose and side effects, and improve the pharmacological efficacy of extracts. Thus, this study evaluated the *in vivo* antidiabetic and antihyperlipidemic activities of ethanolic leaf extracts of *Argyreia pierreana* (AP) and *Matelea denticulata* (MP) plants in comparison with their micellar nanoformulations.
Materials and methods: The mixed micelles (MMs) loaded with crude extracts (CEs) of AP and MD (AP-MMs and MD-MMs) were prepared using a film dispersion technique. Type 2 diabetes was induced in rats using high-fat diet (HFD) and low-dose (35 mg/kg) streptozotocin (STZ) injection. The pharmacological actions of CEs, AP-MMs and MD-MMs were determined in type 2 diabetic Sprague-Dawley rats.
Results: Oral treatments with low-dose AP-MMs and MD-MMs having a mean particle size of 163 ± 10 nm and 145 ± 8 nm respectively, resulted in significantly decreased fasting blood glucose level and increased serum insulin, glucokinase levels, and normalized the elevated levels of hemoglobin A1C and glucose-6-phosphatase. Both extracts significantly decreased serum total cholesterol, triglycerides, and low-density lipoprotein, as well as elevated high-density lipoprotein levels. Additionally, improvements in antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) and malondialdehyde levels were evidenced clearly in tested vital organs (brain, heart, liver).
Conclusion: This is the first report of the antidiabetic and antihyperlipidemic activities of ethanolic leaf extracts of AP and MP plants. Our findings indicate the potential utility of nanotechnology in improving the oral therapeutic efficacy of herbal extracts.

1. Introduction

Metabolic disorders (diabetes and obesity) are a major global health problem; they have an adverse influence on the quality of life and are a leading cause of morbidity in developed countries.[1] The progression of diabetes has been increasing globally from 108 million (1980) to 463 million (2019) with 1.6 million deaths in 2016 attributed to them worldwide, estimated to rise to 578 million (10.2%) by 2030 and 700 million by 2045.[2] According to the WHO, about 80% of the population is using herbal medicines in the aim of treating several diseases[3] and gaining growing attention in global healthcare debates.[4]

There are several supporting pharmacological activities proven for various species of genera *Argyreia* (family Convolvulaceae)[5,6] and *Matelea*[7] (family *Apocynaceae*).[8] In the search for new antidiabetic drugs, for the first time to the best of our knowledge, we have reported the *in vitro* antioxidant and antidiabetic activities of aqueous and ethanolic leaf extracts of two plants belonging to these genera, *Argyreia pierreana*[9] (AP) (Supplementary Fig. 1A) and *Matelea denticulata*[10] (MD) (Supplementary Fig. 1B). Our study

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List of abbreviations

AP	*Argyreia pierreana*
APE	*Argyreia pierreana* extract
APECE	*Argyreia pierreana* ethanolic crude extract
APMME	*Argyreia pierreana* mixed micellar extract
AP-MMs	*Argyreia pierreana* mixed micelles
CAT	Catalase
CEs	Crude extracts
CPCSEA	Committee for the purpose of control and supervision of experiments on animals
DC	Diabetic control
DM	Diabetes mellitus
FBGL	Fasting blood glucose level
GLUT 4	Glucose transporter-4
GSH	Glutathione peroxidase
HbA1C	Glycated heamoglobin
HDL	High-density lipoprotein
HFD	High-fat diet
IAEC	Institutional animal ethics committee
LDL	Low-density lipoprotein
MD	*Matelea denticulata*
MDA	Malondialdehyde
MDE	*Matelea denticulata* extract
MDECE	*Matelea denticulata* ethanolic crude extract
MDMME	*Matelea denticulata* mixed micellar extract
MD-MMs	*Matelea denticulata* mixed micelles
MMEs	Mixed micelle extracts
MMs	Mixed micelles
Na-CMC	Sodium carboxymethyl cellulose
NC	Normal control
NDDSs	Nano drug delivery systems
NPD	Normal pellet diet
P-gp	P-glycoprotein
PPARγ	Peroxisome proliferator-activated receptor gamma
ROS	Reactive oxygen species
SD	Sprague Dawley
SOD	Superoxide dismutase
STZ	Streptozotocin
TC	Total cholesterol
TEM	Transmission electron microscope
TG	Triglyceride's

results revealed the presence of flavonoids, phenols, terpenoids, tannins, alkaloids and glycosides in the extract, with the ethanolic extract exhibiting superior antioxidant and antidiabetic activities than the aqueous extract, this was correlated to the high flavonoid and phenol content in the ethanolic extracts (our previous article).[11] Thus, this study aimed to assess the *in vivo* antidiabetic and antihyperlipidemic activities of ethanolic leaf extracts of *Argyreia pierreana* (AP) and *Matelea denticulata* (MP) plants in comparison with their micellar nanoformulations.

Many studies have shown that herbal medicines (plants crude extracts or isolated biologically active constituents) have good biological activity in *in vitro* assays, but are not reproducible in *in vivo* experiments.[12] Some of the reasons that contribute to the poor *in vivo* efficacy of plant crude extracts (CEs) or isolated constituents are the poor absorption characteristics of the active constituents (flavonoids, tannins, and terpenoids) due to their poor water solubility; their large molecular sizes, which result in a poor diffusion rate across the membrane; and their poor water solubility, which results in reduced dissolution.[13] Furthermore, some molecules are highly sensitive to the acidic pH of the stomach or enzymatic conditions of the gastrointestinal tract and liver (first-pass metabolism) that promotes their destruction and loss of the desired effect after administration.[14]

It has been widely proposed to combine herbal medicines with nanotechnology because nanostructured systems can overcome the above limitations associated with conventional CEs delivery, potentiate the action of plant extracts, reduce the required dose and side effects, and improve the activity.[15–17] Further, nano-drug delivery systems (NDDSs) can reintroduce other components that were discarded, extend a formulation's action, and successfully combine active substances with different degrees of hydrophilicity/lipophilicity.[16] Further, NDDSs can be used to target a specific tissue type of organ.[16] Amongst several nanotechnology-based strategies, the polymeric and co-polymeric nanoparticle systems are the most suitable for screening the antidiabetic and antihyperlipidemic characteristics of herbal extracts or their constituents as these systems are free of lipid excipients.[18] Therefore, in the current study, the amphiphilic block co-polymer-based nanoparticle system (micelles) was selected for oral delivery of very poorly water-soluble ethanolic leaf extracts of the plants AP and MD.

The strategy of applying nanotechnology (nano micellar system) to the plant extracts or their isolated constituents has been widely cited in the literature.[19,20] There are several studies reporting enhanced oral absorption and bioavailability of various chemicals such as using nano micellar delivery system.[15–17] For instance, Choongjin Bane and co-workers have proven the enhanced oral bioavailability of curcumin micelles stabilized by PEG10SE and PEG100S.[19] Similarly, Yuan Zhu and co-workers increased the oral bioavailability of capsaicin using simple mixed polymeric micelles.[20] In this study, by considering the above copolymer-based delivery systems and their simple development procedure, we have developed a Poloxamer 407 and tween-80 based nano mixed micellar system to encapsulate poorly water-soluble leaf extracts of the plants AP and MD to determine their antidiabetic characteristics in comparison with their crude extracts.

2. Materials and methods

2.1. Drugs and reagents

Metformin and Streptozotocin were gifted to us by Dr. Reddy's, Laboratory, Hyderabad, India, and Sisco Research Laboratories Pvt. Ltd., Hyderabad, respectively. Citric acid, Sodium citrate, Cholesterol, Yeast powder, Disodium hydrogen orthophosphate, Sodium Carboxymethylcellulose, Sodium chloride, and Formalin were procured from S D Fin-Chem Ltd., Mumbai, India. 10% Chloral hydrate (Sigma-Aldrich, Mumbai, India), NPD-Nutrimi STD1020 (Nutrivet Life Sciences, Pune, India), Casein (Eric India Agro& Food Pvt. Ltd., Bikaner, India), Vitamin and mineral mix (Henry's Healthy Pets, Floyd, Virginia, USA) and DL-methionine (Chemkart, Bhiwandi, Maharashtra, India) were also procured and used in the study.

2.2. Collection of the plants

Argyreia pierreana (AP) and *Matelea denticulata* (MD) plants were collected and authenticated by Dr. Madhava Chetty, Department of Botany, SV University, Tirupathi, India (Voucher number 1364 and 1596).

Table 1
Effect of high dose crude extracts and low dose mixed micellar extracts on FBGL of diabetic rats.

Group	Treatment	FBGL (mmol/L)				
		0 day	7 day	14 day	21 day	28 day
NC-1	0.5% Na-CMC (10 mL/kg)	4.49 ± 0.44	4.27 ± 0.30	4.35 ± 0.45	4.49 ± 0.45	4.29 ± 0.35
NC-2	Polymer vehicle (10 mL/kg)	4.73 ± 0.24	4.79 ± 0.30	4.68 ± 0.26	4.84 ± 0.20	4.72 ± 0.39
DC	Diabetic + Na-CMC (0.5%)	16.35 ± 1.35	17.97 ± 0.88	18.83 ± 1.79	20.20 ± 2.32	22.60 ± 1.81
STD	Diabetic + Metformin (500 mg/kg)	16.53 ± 1.39	14.47 ± 1.59[a]	12.32 ± 1.13[a]	9.66 ± 0.61[a]	6.22 ± 1.06[a]
Test-1	Diabetic + APECE (400 mg/kg)	17.41 ± 1.57	15.42 ± 1.13[c]	16.55 ± 1.32[c]	17.70 ± 1.34[c]	17.51 ± 1.83[a]
Test-2	Diabetic + MDECE (400 mg/kg)	16.96 ± 2.00	16.61 ± 1.78[c]	16.02 ± 1.98[b]	16.91 ± 1.48[a]	16.17 ± 2.02[a]
Test-3	Diabetic + APMME (200 mg/kg)	17.48 ± 1.48	14.63 ± 2.09[a]	12.67 ± 0.93[a]	9.50 ± 1.02[a]	6.01 ± 1.02[a]
Test-4	Diabetic + MDMME (200 mg/kg)	17.07 ± 2.48	14.30 ± 1.86[a]	11.97 ± 1.96[a]	9.00 ± 0.94[a]	5.55 ± 0.90[a]

Values are Mean ± SD; n = 6. [c]p < 0.05, [b]p < 0.01, [a]p< 0.001 vs. diabetic control (DC). APECE = Argyreia pierreana ethanolic crude extract; MDECE = Matelea denticulata ethanolic crude extract; APMME = Argyreia pierreana mixed micellar extract; MDMME = Matelea denticulata mixed micellar extract.

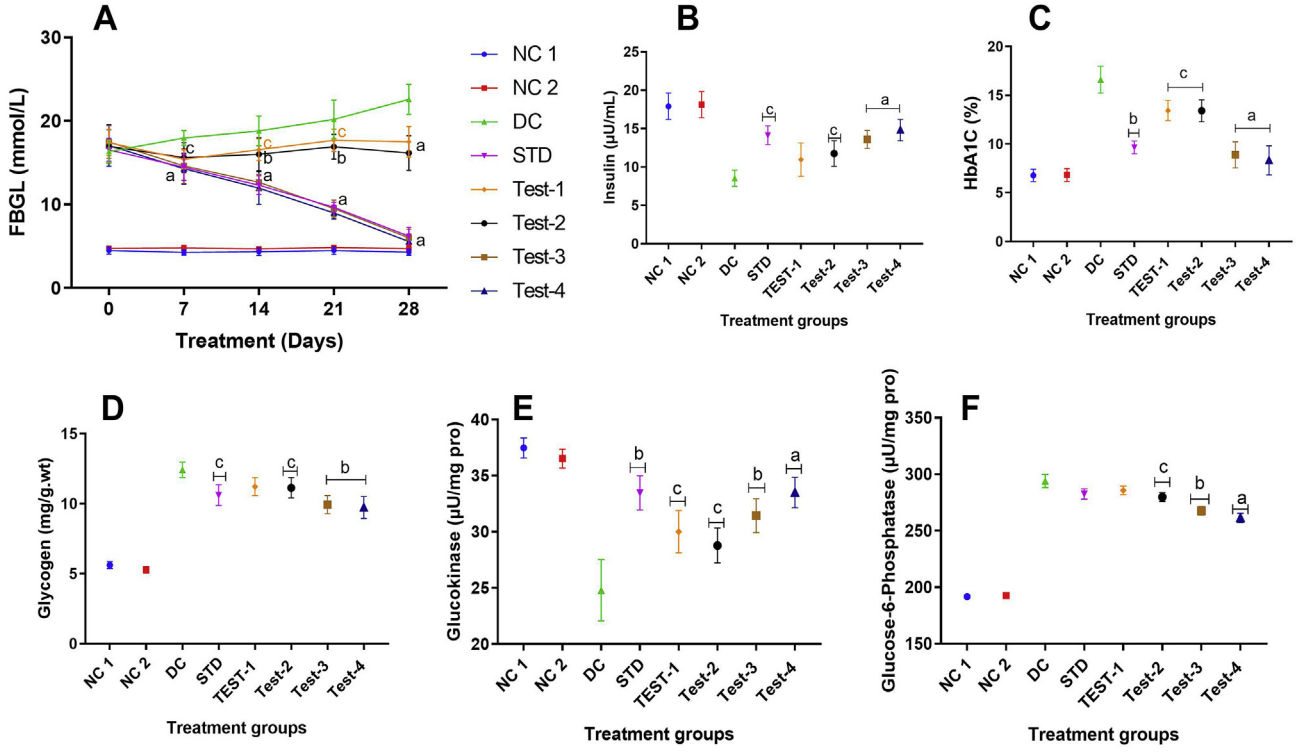

Fig. 1. Effect of high dose crude extracts and low dose mixed micellar extracts on diabetic parameters. Values are Mean ± SD, n = 6. [c]p < 0.05, [b]p < 0.01 and [a]p< 0.001 vs. diabetic control (DC).

Table 2
Effect of high dose crude extracts and low dose mixed micellar extracts on metabolic parameters of diabetic rats.

Group	Treatment	Parameters				
		Insulin (µU/ml)	HbA1C (%)	Glucokinase (µU/mg pro)	Glucose-6-Phosphatase (µU/mg pro)	Hepatic glycogen (mg/g)
NC-1	0.5% Na-CMC (10 mL/kg)	17.9 ± 1.72	6.78 ± 0.63	37.5 ± 0.88	192.±1.45	5.62 ± 0.25
NC-2	Polymer vehicle (10 mL/kg)	18.1 ± 1.72	6.82 ± 0.66	36.5 ± 0.84	193 ± 1.47	5.27 ± 0.22
DC	Diabetic + Na-CMC (0.5%)	8.53 ± 1.06	16.6 ± 1.37	24.8 ± 2.73	294 ± 5.89	12.4 ± 0.55
STD	Diabetic + Metformin (500 mg/kg)	14.1 ± 1.23[b]	9.65 ± 0.65[b]	33.5 ± 1.52[b]	283 ± 4.43[b]	10.6 ± 0.74[c]
Test-1	Diabetic + APECE (400 mg/kg)	11.0 ± 2.18	13.4 ± 1.03[c]	30.0 ± 1.88[c]	286 ± 3.79	11.2 ± 0.64
Test-2	Diabetic + MDECE (400 mg/kg)	11.8 ± 1.67[c]	13.4 ± 1.13[c]	28.8 ± 1.55[c]	280 ± 3.81[c]	11.1 ± 0.73[c]
Test-3	Diabetic + APMME (200 mg/kg)	13.6 ± 1.17[a]	8.89 ± 1.34[a]	31.4 ± 1.51[b]	268 ± 3.81[b]	9.93 ± 0.64[b]
Test-4	Diabetic + MDMME (200 mg/kg)	14.8 ± 1.39[a]	8.83 ± 1.49[a]	33.5 ± 1.36[a]	261 ± 3.97[a]	9.74 ± 0.77[b]

Values are Mean ± SD; n = 6. [c]p < 0.05, [b]p < 0.01, [a]p< 0.001 vs. diabetic control (DC). APECE = Argyreia pierreana ethanolic crude extract; MDECE = Matelea denticulata ethanolic crude extract; APMME = Argyreia pierreana mixed micellar extract; MDMME = Matelea denticulata mixed micellar extract.

2.3. Animals

Male Sprague-Dawley (SD) rats (160–180 g) were procured from VAB BioSciences, Medipally, Ghatkesar, Hyderabad (Reg.No. 282/PO/RcBt/S2000/CPCSEA). The animals were housed in standard polypropylene cages (six rats/cage) and maintained under controlled room temperature (22±2 °C) and humidity (55 ± 5%) with a 12 h light and 12 h dark cycle. All the rats were provided with commercially

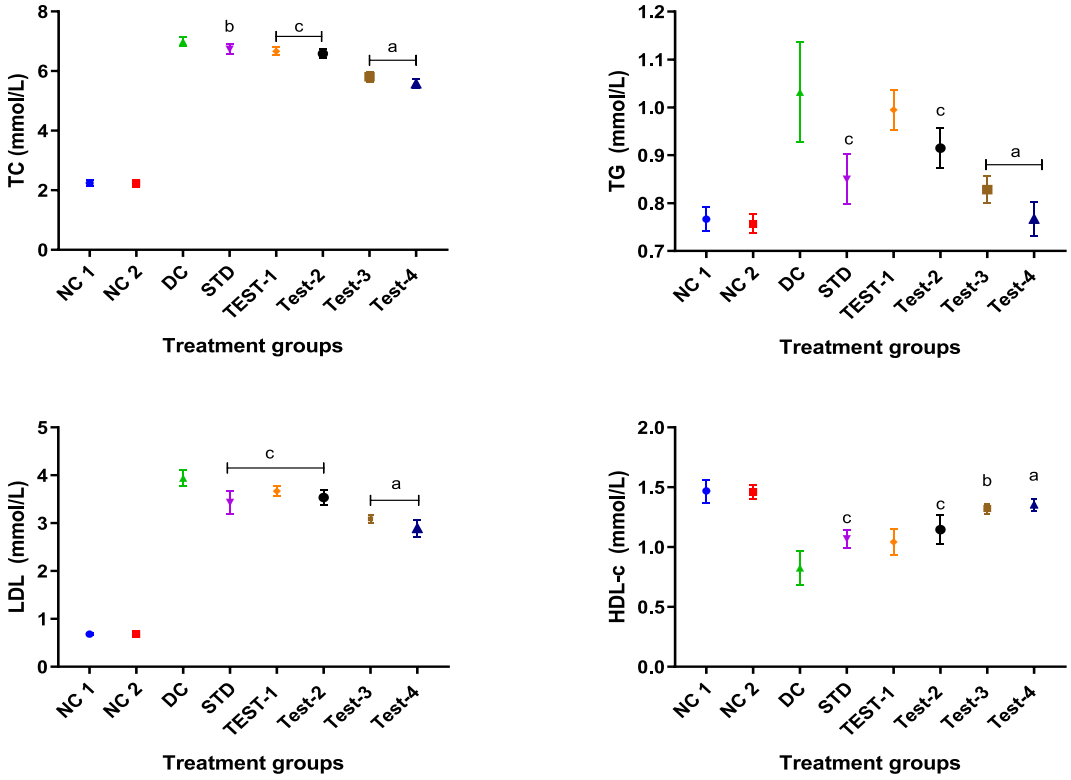

Fig. 2. Effect of high dose crude extracts and low dose mixed micellar extracts on lipid profile. Values are Mean ± SD, n = 6. $^{c}p < 0.05$, $^{b}p < 0.01$ and $^{a}p < 0.001$ vs. diabetic control (DC).

available rat normal pellet diet (NPD) and water *ad libitum*, before the dietary manipulation. The guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), Govt. of India were followed during the experiment. The prior permission was sought from the institutional animal ethics committee (IAEC) of SSJ College of Pharmacy, V. N Pally, Hyderabad for conducting the study (Reg.No.1488/PO/Re/S/11/CPCSEA/12/2018).

2.4. Preparation of extracts

The plant leaves were congregated, cleaned, and dried in a shady atmosphere for one week. The ethanolic (90%) extract was prepared by using a soxhlet apparatus at 60-75 °C for 4 h. The ethanolic extract was collected in Petri plates, dried under abridged temperature (40 °C) in a hot air oven. The prepared extracts were then stored under 20 °C until needed for further study.[21,22]

2.5. Preparation of crude extract-loaded mixed micelles (MMs)

MMs loaded with extract were prepared by the film dispersion method.[23] The compositions of MMs (Supplementary Tables 1 and 2) were dissolved in 10 mL of methanol using a bath sonicator for 5 min. The methanol was evaporated at room temperature, and the film formed at the beaker bottom was dispersed with 10 mL of double distilled water using a bath sonicator for 5 min. The obtained mixed micellar solutions containing extracts were visually inspected for complete solubilization of extract and observations were reported accordingly in the Supplementary Tables 1 and 2

2.6. Mean particle size determination

The mean particle size of the optimized extract-loaded MMs was determined using Malvern ZS Zetasizer (Ver. 6.20 Malvern Instruments Ltd). All measurements were performed in triplicate at 25 °C.[24,25]

2.7. Confirmation of co-polymer aggregates (micellar system)

The surface morphology of the prepared optimized MMs was determined using a transmission electron microscope (TEM, FEI Tecnai T-20ST). Briefly, the mixed micellar solution was placed on the copper grid and air-dried. Then 2% w/v phosphotungstic acid solution (negative staining) was added, air-dried overnight and observed under TEM.[24,25]

2.8. Determination of antidiabetic, antihyperlipidemic and antioxidant activities

2.8.1. Induction of type 2 diabetes

The rats were divided into two groups for two different dietary regimens for the initial period of 2 weeks. One group was fed with a dietary regimen consisting of NPD (12% of calories as fat) and the other group was fed with a high-fat diet (HFD) (58% fat, 25% protein, and 17% carbohydrate, as a percentage of total kcal) *ad libitum*.[26] The composition of HFD per kilogram of rat includes; powdered NPD (Nutrimix-Std 1020) 365 g, lard 310 g, casein 250 g, cholesterol 10 g, vitamin and mineral mix 60 g, DL-Methionine 03 g, yeast powder 1 g and sodium chloride 1 g. After 14 days of nutritional modification, the rats of the group fed with HFD were administered a single low dose of streptozotocin (STZ, 35 mg/kg) intraperitoneally (i.p.) while the control rats were administered a citrate buffer (pH 4.4) in a dose size of 1 mL/kg (i.p). After 3 days, the rats were subjected to fasting blood glucose level (FBGL) measurement using the Accu-Check Active glucometer (Roche Diabetes Care, GmbH Sandhofer Strasse, Mannheim, Germany). The rats which showed FBGL in the range of 10–30.0 mmol/L were considered as diabetic

Table 3
Effect of high dose crude extracts and low dose mixed micellar extracts on oxidative stress in vital organs of diabetic rats.

Group	Treatment	Brain				Heart				Liver			
		SOD (μg/mg)	CAT (μg/mg)	GSH (μg/mg)	MDA (nmol/g)	SOD (μg/mg)	CAT (μg/mg)	GSH (μg/mg)	MDA (nmol/g)	SOD (μg/mg)	CAT (μg/mg)	GSH (μg/mg)	MDA (nmol/g)
NC-1	0.5% Na-CMC (10 mL/kg)	19.5 ± 0.51	11.1 ± 0.18	19.5 ± 0.51	32.9 ± 1.20	18.6 ± 0.68	11.1 ± 0.66	3.56 ± 0.30	34.9 ± 0.95	17.8 ± 0.28	9.29 ± 0.25	3.15 ± 0.06	17.8 ± 0.28
NC-2	Polymer vehicle (10 mL/kg)	19.7 ± 0.45	11.0 ± 0.39	19.7 ± 0.45	32.7 ± 2.17	18.5 ± 0.53	11.0 ± 0.30	3.11 ± 0.20	34.3 ± 2.02	17.8 ± 0.77	9.19 ± 0.26	3.18 ± 0.10	17.6 ± 0.77
DC	Diabetic + Na-CMC (0.5%)	8.04 ± 0.68	6.06 ± 0.28	8.04 ± 0.68	92.2 ± 2.39	7.69 ± 0.80	4.95 ± 0.46	1.41 ± 0.34	99.6 ± 2.67	9.80 ± 0.69	5.01 ± 0.59	1.47 ± 0.28	20.80 ± 1.13
STD	Diabetic + Metformin (500 mg/kg)	9.63 ± 0.37^{c}	6.80 ± 0.41^{ns}	9.63 ± 0.36^{c}	88.5 ± 2.33^{ns}	9.08 ± 0.92^{c}	6.01 ± 0.56^{c}	1.98 ± 0.46^{c}	94.8 ± 2.67^{ns}	12.±1.11^{c}	6.42 ± 0.64^{ns}	1.97 ± 0.19^{c}	19.0 ± 1.00^{ns}
Test-1	Diabetic + APECE (400 mg/kg)	10.3 ± 0.91^{c}	7.16 ± 0.50^{c}	10.3 ± 0.91^{c}	82.6 ± 3.35^{c}	9.17 ± 0.49^{c}	5.98 ± 0.83^{c}	1.96 ± 0.27^{c}	95.2 ± 4.24^{ns}	12.0 ± 0.73^{c}	6.17 ± 0.89^{ns}	2.07 ± 0.15^{c}	18.9 ± 0.73^{ns}
Test-2	Diabetic + MDECE (400 mg/kg)	11.1 ± 0.87^{c}	7.28 ± 0.70^{c}	11.1 ± 0.87^{c}	82.2 ± 2.78^{c}	9.33 ± 0.93^{b}	6.0 ± 0.74^{c}	1.99 ± 0.24^{c}	93.7 ± 4.15^{c}	12.4 ± 1.35^{c}	6.70 ± 0.79^{c}	1.92 ± 0.10^{c}	18.7 ± 0.94^{c}
Test-3	Diabetic + APMME (200 mg/kg)	15.2 ± 1.10^{a}	8.79 ± 0.32^{a}	15.4 ± 1.14^{a}	54.8 ± 2.78^{a}	14.2 ± 1.1^{a}	8.92 ± 0.30^{a}	2.56 ± 0.35^{a}	64.6 ± 3.54^{a}	13.9 ± 0.66^{a}	7.94 ± 0.73^{a}	2.60 ± 0.18^{a}	18.2 ± 0.66^{c}
Test-4	Diabetic + MDMME (200 mg/kg)	16.7 ± 1.03^{a}	9.81 ± 0.66^{a}	15.8 ± 1.04^{a}	49.9 ± 1.85^{a}	15.3 ± 0.92^{a}	9.80 ± 0.15^{a}	2.82 ± 0.21^{a}	60.3 ± 3.60^{a}	14.1 ± 0.80^{a}	8.32 ± 0.65^{a}	2.91 ± 0.18^{a}	17.9 ± 0.57^{b}

Values are Mean ± SD; n = 6. ns = non significance, ^{c}p< 0.05, ^{b}p < 0.01, ^{a}p< 0.001, vs. diabetic control (DC). APECE = *Argyreia pierreana* ethanolic crude extract; MDECE = *Matelea denticulata* ethanolic crude extract; APMME = *Argyreia pierreana* mixed micellar extract; MDMME = *Matelea denticulata* mixed micellar extract.

and selected for the further pharmacological screening of CEs and their micellar formulations.

2.8.2. Treatment schedule

The animals were divided randomly into 8 groups and six animals were taken in each group (n = 6) and treated orally once a day for 28 days as follows:

The normal control group-1 (NC-1) and normal control group-2 (NC-2) each received 0.5% sodium carboxymethyl cellulose (Na-CMC) and blank copolymer vehicle (bank MMs) at a dose volume of 10 mL/kg respectively. The diabetic control group (DC) and standard treatment group (STD) were treated with 0.9% normal saline (10 mL/kg) and metformin (500 mg/kg) respectively. The test-1 and test-2 groups received CEs (APECE and MDECE, 400 mg/kg) prepared in 0.5% Na-CMC respectively. The test-3 and test-4 groups were treated with the mixed micellar extract (MME) of AP (APMME) and MD (MDMME) at a dose volume (10 mL/kg) equivalent to 200 mg/kg AP and MD respectively for 28 days. All group rats were fed with their respective diet until the end of the study.

2.9. Biochemical and tissue parameter analysis

The FBGL was determined at 0, 7, 14, 21, and 28 day using an Accu-Check Active glucometer. On the last day of the study, the rats were anesthetized using 10% chloral hydrate (0.3 mL/100 g),[27] and their blood was collected into centrifugation tubes by cardiac puncture and allowed to clot at room temperature for 10 min. The tubes were then centrifuged at 4 °C and 3000 rpm for 20 min. The serum was collected and stored in a deep freezer (−20 °C) until further analysis. The serum was analyzed for insulin, hemoglobin A1C (HbA1C), and metabolic parameters such as glucokinase, glucose-6-phosphatase, and hepatic glycogen using commercial biochemical kits. Further, the serum samples were analyzed for their lipid profile (TC: total cholesterol, TG: triglycerides, LDL: low-density lipoprotein, HDL: high-density lipoprotein). Post blood collection, the animals were quickly dissected, vital organs (brain, heart, and liver) were isolated and analyzed for the activities of antioxidant enzymes such as SOD (superoxide dismutase), CAT (catalase), GSH (glutathione peroxidase), and the MDA (malondialdehyde) level. In addition, the pancreas was removed, suffused using 0.9% normal saline, and then placed in 10% formalin solution for preservation until histopathological examination.

2.10. Histopathology of the pancreas

The pancreas was processed and embedded in paraffin for a thin section using a microtome (Leica, Japan). The section cuttings of 5-μm thickness were stained with hematoxylin and eosin. Then examined under the microscope (Zeiss Microscope, India).[28]

2.11. Statistical analysis

The results obtained were analyzed by one-way ANOVA followed by Dennett's test using GraphPad Prism 8.0.1 and expressed as mean ± SD. The value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Preparation of MMs loaded with crude extract

The solubility of AP and MD extracts (APE and MDE) in presence of different concentrations of co-polymers (Poloxamer 188 and Poloxamer 407) and an amphiphilic surfactant, tween-80 are

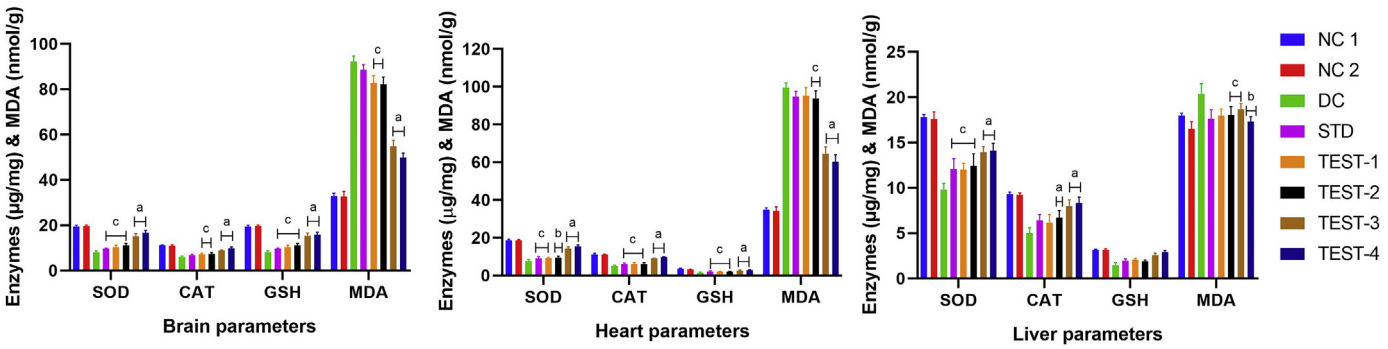

Fig. 3. Effect of high dose crude extracts and low dose mixed micellar extracts on oxidative stress in vital organs homogenate of diabetic rats. Values are Mean ± SD, n = 6. $^{c}p < 0.05$, $^{b}p < 0.01$ and $^{a}p < 0.001$ vs. diabetic control (DC).

presented in Supplementary Tables 1 and 2 The extracts were found to have moderately solubility in the presence of copolymer Poloxamer 407 and tween-80 rather than Poloxamer188 and tween-80. The AP extract was found to be completely soluble at APE:Poloxamer 407:Tween-80 wt ratio of 1:4:4 (Supplementary Fig. 2A), whereas MD was found to be completely soluble at MDE:Poloxamer 407:Tween-80 wt ratio 1:3:3 (Supplementary Fig. 2A). These obtained results indicate that the AP is less soluble in the aqueous phase than MD and therefore requires more copolymer concentration to remain completely soluble. However, in the present study, both AP-MMs and MD-MMs prepared at Extract:Poloxamer 407:Tween 80 ratio of 1:4:4 and 1:3:3 are used in *in vivo* characterization.

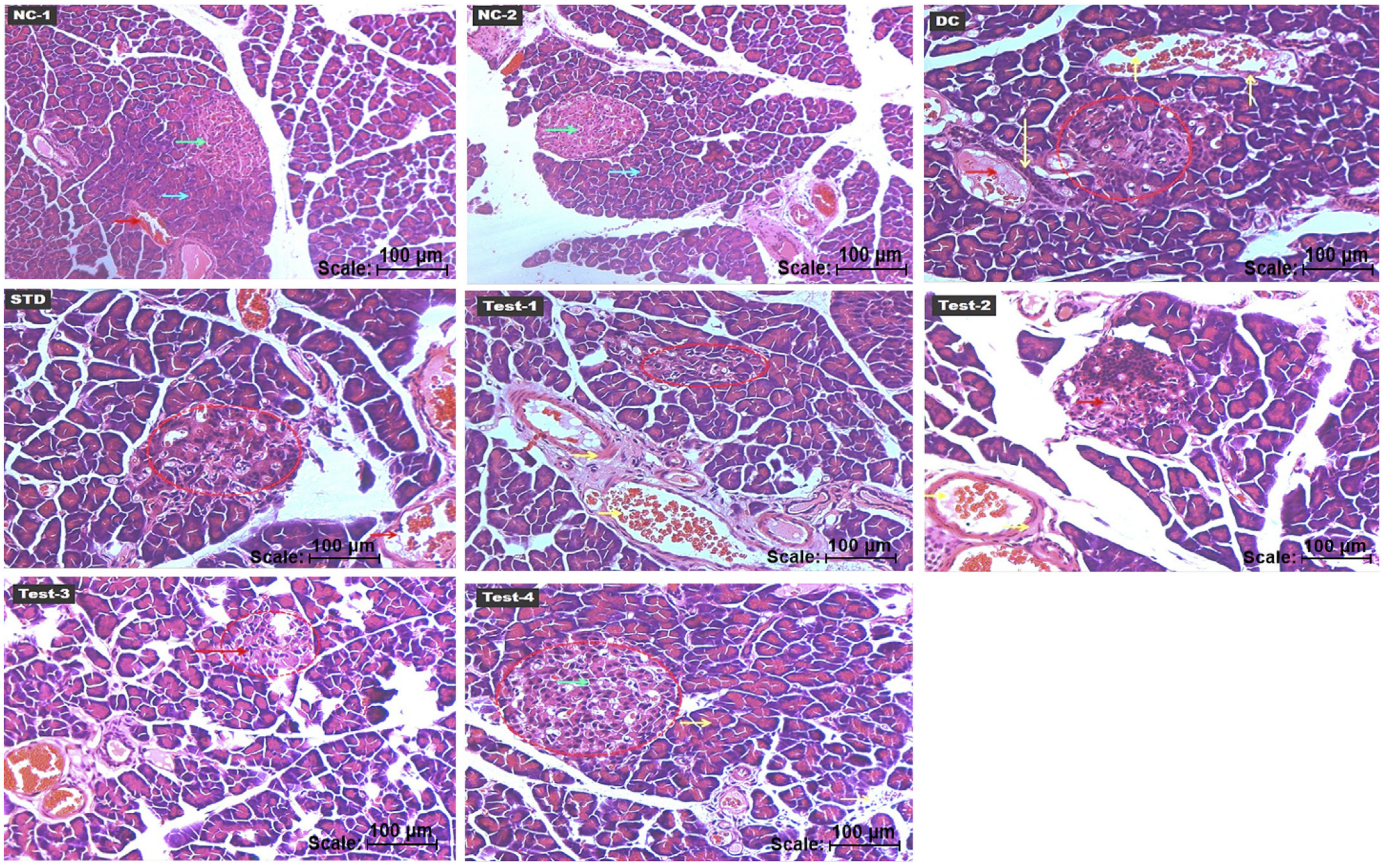

Fig. 4. Microphotographs of histopathological changes in pancreas at 200× magnification of different treatment groups: NC-1 and NC-2: Beta cells (green arrow), acinar cells (light blue arrow) are appeared normal and normal ductular pancreas with secretion (red arrow); DC: Atrophy of islets with necrosis, fibrosis and apoptosis of beta cells (red circle), ductular degeneration (red arrow), and ductular degeneration (orange arrow); STD: Degeneration, necrosis and fibrosis of beta cells with atrophy of islets (red circle), tubular degeneration (red arrow); Test-1: Degeneration of beta cells with atrophy (red circle), degeneration and necrosis of beta cells (red arrow), ductular fibrosis and degeneration (yellow arrow); Test-2: Degeneration, fibrosis and atrophy of islets (red arrow), mild ductular degeneration with fibrosis (yellow arrow); Test-3: Mild regeneration with preserved beta cells of beta cells (red arrow), acinar cells are normal (green arrow); Test-4: Normal beta cells (green arrow) with mild ductular degeneration and fibrosis (red arrow) and normal acinar cells (light blue arrow).

3.2. Mean particle size determination

The mean particle size of AP-MMs and MD-MMs are presented in Supplementary Fig. 2. The AP-MMs (1:4:4 wt ratio) showed a moderately increased mean particle size of 163 ± 10 nm (Polydispersity index, PDI: 0.271 ± 0.07) (Supplementary Fig. 2B) as compared to MD-MMs (1:3:3 wt ratio) which showed a mean particle size of 145 ± 8 nm (PDI: 0.226 ± 0.08) (Supplementary Fig. 2C). This moderately increased mean particle size of AP-MMs might be due to its high copolymer concentration as compared to MD-MMs.

3.3. Confirmation of co-polymer aggregates (micellar system) using TEM

The TEM image of AP-MMs (Supplementary Fig. 2D) confirmed the aggregate (micelle) formation. Further, it is very clear from the image that the formed micellar system is almost homogenous, moderately aggregated, and spherical in shape.

3.4. Effect of crude and micellar extracts on blood glucose levels in diabetic rats

The antidiabetic characteristics of CEs and mixed micellar extracts (MMEs) in diabetic rats were determined by measuring FBGL of rats after every week throughout the treatment period of 28 days (Table 1 and Fig. 1A). In the non-diabetic control groups (NC-1 and NC-2), the FBGL was almost the same throughout the treatment period indicating no effect of Na-CMC and blank copolymer vehicle on FBGL. The FBGL was found to be significantly higher in DC group rats and increased over time. The metformin treatment (STD) resulted in significantly decreased FBGL (after 7 days of treatment) and was similar to the FBGL of non-diabetic rats at the end of the study.

The effect of ethanolic crude extracts of AP and MD (APECE and MDECE), at a dose of 400 mg/kg, on reducing FBGL was found to be significant when compared to the DC group, however the effect was found to be less ($p < 0.05$) when compared to standard metformin treatment ($p < 0.001$). Furthermore, the MDECE was moderately more effective than the APECE after 14 days of treatment. Surprisingly, treatment with APMME and MDMME resulted in a significantly decreased FBGL ($p < 0.001$) at a dose half of the CEs tested (200 mg/kg). The APMME and MDMME showed hypoglycemic activities comparable to those of standard metformin treatment at a dose of 200 mg/kg which is again significantly more effective than the standard metformin dose (500 mg/kg) and CEs dose (400 mg/kg). Further, the hypoglycemic effect of MDMME was found to be moderately higher than the APMME on the 28th day of the study.

3.5. Effect of crude and mixed micellar extracts on metabolic parameters

The effect CEs and MMEs on metabolic parameters such as serum insulin level, HbA1C, glucokinase, glucose-6-phosphatase, and hepatic glycogen are represented in Table 2 and Fig. 1B–F and. The MDECE treatment caused a significant increase in serum insulin levels compared to the DC group, however, the effect is moderately less significant than standard metformin treatment. Surprisingly, the low dose of APMME and MDMME treatments resulted in significantly increased insulin levels, compared to high dose CEs. Further, the effect of MMEs on serum insulin levels was found to be moderately higher than that of standard metformin.

The effect of CEs in normalizing the elevated glycated hemoglobin (HbA1C) level in diabetic rats was found less significant than the standard metformin treatment. However, their low dose micellar form normalized the elevated HbA1C level more effectively than standard metformin.

Similarly, the effect of CEs and MMEs in restoring the serum glucokinase level was found to be significant when compared to the DC group. Again, the low dose MMEs significantly restored the glucokinase level compared to high dose CEs and metformin. The MDMME treatment was found to be more effective than all other treatments. Likewise, the MDMME treatment was found to be significantly more effective than APMME, CEs, and metformin in restoring serum glucose-6-phosphatase levels. The diabetic rats showed an elevated level of liver glycogen. The low dose MMEs significantly reduced the elevated glycogen level when compared to CEs and metformin.

3.6. Effect of crude and micellar extracts on lipid profile

The effect of high dose CEs and low dose MMEs on the serum lipid profile is presented in Fig. 2 and Supplementary Table 3. The metformin, MDECE, and MMEs showed significantly reduced serum TC, TG, LDL, and Increased HDL levels when compared to the DC group. The APECE treatment caused no significant effects on serum TG and HDL levels however its treatment significantly reduced the serum TC and LDL levels compared to the DC group. The treatment with low dose MMEs showed a significantly higher antihyperlipidemic activity (reduced serum level of TC, TG, LDL, and Increased HDL) when compared to high dose CEs and metformin treatments.

3.7. Effect of crude and micellar extracts on MDA level and antioxidant enzymes levels in vital organs

In the current study, the antioxidant activities of high dose CEs versus low dose MMEs are determined by measuring the MDA levels and activities of antioxidant enzymes (SOD, CAT, and GSH) in vital organs of the diabetic rats after 28 days of treatment (Table 3 and Fig. 3). The MDA levels in the brain and heart of the DC group were found to be significantly higher compared to the non-diabetic group, whereas its level in the liver is significantly less than non-diabetic rats. The metformin treatment does not cause any significant effects on brain heart and liver MDA levels. The effect of low dose MMEs on the MDA level of all vital organs was found to be highly significant compared to high dose CEs. Additionally, the MDMME treatment caused a conspicuous increase in the MDA level compared to APMME.

The induction of diabetes in rats (DC) resulted in a significantly lower level of antioxidant enzymes in all vital organs compared to non-diabetic rats indicating a high level of oxidative stress in the vital organs of diabetic rats. The standard metformin and high dose CEs resulted in almost similar effects against all enzymes in all organs. However, the effect of MDECE was found to be highly significant against certain enzymes and in some organs. The effect of low dose MMEs in restoring all antioxidant enzyme levels in all vital organs was found to be highly significant when compared to DC, metformin, and high dose CEs groups. In addition, the MDMME treatment showed a conspicuous increase in all antioxidant enzyme activities in all vital organs as compared to APMME.

3.8. Effect of crude and micellar extracts on the pancreas

The histological changes occurred in the diabetic rat's pancreas, whereas no histological changes were seen in normal rats. The low dose mixed micellar extracts (MMEs) treatment normalized the histological changes effectively compared to high dose CEs treatment (Fig. 4).

4. Discussion

The polymeric MMs can be prepared from two or more chemically different copolymers. Based on this rationale, the MMs have distinctive advantages over conventional individual micelles (prepared from single copolymer) by demonstrating increased aqueous solubility, enhanced stability, prolonged circulation time, improved pharmacokinetic and dynamic properties of drugs.[29]

In the current study, the Poloxamer 407, a synthetic amphiphilic block copolymer composed of poly (ethylene oxide) (PEO) and poly (propylene oxide) (PPO) blocks arranged in a triblock structure PEO-PPO-PEO, that self-assembles into micelles in aqueous solution, is used for solubilization. The hydrophobic PPO segments form the hydrophobic inner core which ensures high drug loading, and the hydrophilic PEO segments form a hydrophilic outer shell that can avoid the recognition and elimination by the reticuloendothelial system.[30] Another amphiphilic surfactant used in the present study is tween-80, a widely used surfactant for efficient delivery of poorly soluble substances, which self-assembles into micelles in aqueous media.[31]

In the current study, the polymeric MMs were composed of chemically different copolymers, Poloxamer 407 and Tween-80, which were developed for improved oral delivery of water-insoluble crude ethanolic extracts of the leaves of plants AP and MD. The developed mixed micellar formulations were characterized for mean particle size and micelle formation in accordance with the previous report.[23,24] and the TEM results confirmed the self-assembling nature of Poloxamer 407 and tween 80 in water into mixed micellar extracts.[24,25]

Diabetes mellitus is a prevalent disease worldwide, it is a chronic endocrine metabolic disease and about 97% of diabetic patients suffer from type 2 DM. It is characterized by hyperglycemia and impaired glucose metabolism that leads to an increase in lipid and lipoprotein levels as well as free radical production. Meanwhile, free radicals can initiate peroxidation of lipids, which in turn stimulates protein glycation, and oxidative stress.[27]

The lack of multiple effects of clinically approved antidiabetic drugs (insulin and synthetic drugs) on lipid profiles and antioxidant stress[32] resulted in extensive research into traditional herbal medicines which may play an important role in multiple effects. Therefore, the current study was aimed at the development of herbal medicines (ethanolic leaf extracts of AP and MD) for multiple pharmacological effects, antidiabetic, antihyperlipidemic, and antioxidant in type 2 DM rats.

The diabetic rat model induced by HFD and low dose STZ, as this model shows metabolic characteristics of the type 2 DM similar to humans[26] to study the concurrent multiple pharmacological activities for extracts. The hypoglycemic activity of CEs and their micellar forms were determined by directly measuring the FBGL and metabolic parameters. The elevated levels of FBGL, HbA1C, glucose-6-phosphatase, glycogen and decreased levels of insulin and glucokinase were noticed in diabetic rats. A regular treatment of diabetic rats with high dose CEs and low dose MMEs remarkably restored the above changes.

The *in vivo* antidiabetic activity of the extracts observed in the current study could be correlated to the *in vitro* study results wherein the increased glucose uptake by rat skeletal muscle (L6) cells via GLUT-4 and PPARγ expression was observed[11] (Supplementary Fig. 3). In addition, the antidiabetic activity of the extracts observed might be due to the presence of phytochemicals such as flavonoids, phenols, and terpenoids confirmed by the preliminary phytochemical screening[11] which are generally claimed to be beneficial for their antidiabetic activity in accordance with previous reports.[33–36]

In this study, we observed significantly increased glucokinase and insulin levels with MMEs treatment over CEs treatment (Table 2, Fig. 1B). The increased insulin level might be due to the stimulation of glucokinase which causes glucose phosphorylation and leads to ATP-sensitive K^+ channel depolarization dependent Ca^{+2} influx in β-cells. The increased cytosolic Ca^{+2} causes the migration, fusion, and fluidization of insulin stored vesicles and cell membrane which leads to exocytosis of insulin[37] (Supplementary Fig. 4). Further, the *in vitro* free radical scavenging property (noticed in *in vitro* study[11]) and *in vivo* tissues antioxidant properties of the extracts might assist in the prevention of oxidative damage resulting in β-cell regeneration and increased insulin release.

In uncontrolled type 2 DM, the disorders of lipid metabolism are associated with decreased HDL and increased TG, TC, and LDL.[38] In the present study, a marked increase in serum lipids and lipoproteins is observed in diabetic rats and the regular treatment of high dose CEs and low dose MMEs significantly restored the serum lipid profile. Thus, the treatment with low dose MMEs may significantly reduce the risk factor for coronary heart diseases through improving lipid metabolism. The PPARγ gene expression is attributed to the inhibition of hepatic apolipoprotein C-III expression and increases the lipoprotein lipase activity which results in a reduction in TG production, promotion of the plasma TG clearance and increases in HDL levels.[39] The treatment with extracts caused the increased expression of the PPARγ gene in L6 cells *in vitro*.[11] Thus, *in vivo* antihyperlipidemic activity of the extracts might be correlated to their effect on PPARγ gene expression. Further, the antihyperlipidemic activity of extracts might be attributed to the presence of flavonoids and phenols in accordance with the previous reports.[40,41]

The elevated blood glucose level (hyperglycemia) in diabetes facilitates the free radicals production and depletes the natural antioxidants.[42] The SOD, CAT, and GSH are important enzymes that scavenge the free radicals and protect the cells against oxidative stress injury.[43] In addition, the increase of MDA content in diabetic rats reflects the degree of lipid peroxidation.[44] In the present study, the decreased level of SOD, CAT, and GSH and elevated levels of MDA in all vital organs of the diabetic rats indicated the high level of oxidative stress. The low dose MMEs significantly improved the SOD, CAT, and GSH and lowered the MDA levels. The observed *in vivo* antioxidant activity of the extracts is might be correlated to the presence of supporting phytochemicals such as flavonoids and phenols which can scavenge the free radicals[11] and stimulating the property of antioxidant enzymes (Table 3, Supplementary Fig. 5). These phytochemicals are commonly claimed to have antioxidant activities in accordance with earlier reports.[13,15]

5. Conclusion

This study explored, for the first time to the best of our knowledge, the *in vivo* antidiabetic and antihyperlipidemic activities of ethanolic leaf extracts of AP and MP plants in HFD-STZ induced type 2 diabetic SD rats. Furthermore, the study results revealed significantly higher antidiabetic, antihyperlipidemic, and tissue antioxidant activities of micellar nanoformulations (at a dose half of the dose tested for crude extracts). Moreover, the micellar nanoformulations of MD leaf extracts have shown moderately higher activities over micellar nanoformulations of AP leaf extracts. These obtained results indicated that the entrapment of the crude extracts in the hydrophobic core of nano micelles could significantly improve their oral efficacy, and thus combining herbal medicine with nanotechnology could be a potential approach for improved oral delivery of herbal extracts/drugs.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2020.08.001.

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