Bulletin of Faculty of Pharmacy, Cairo University 56 (2018) 169–174

Cairo University
Faculty of Pharmacy

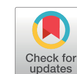
Contents lists available at ScienceDirect

Bulletin of Faculty of Pharmacy, Cairo University

journal homepage: www.elsevier.com/locate/bfopcu

BULLETIN OF FACULTY OF PHARMACY, CAIRO UNIVERSITY

Atherogenicity of diabetic rats administered single and combinatorial herbal extracts



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ARTICLE INFO

Keywords:
Atherogenicity
Diabetes mellitus
Dyslipidemia
Herbal
Serum lipid profile

ABSTRACT

Objective: The present study sought to investigate atherogenicity of alloxan-induced diabetic rats administered single and combinatorial herbal formulations of *Acanthus montanus*, *Asystasia gangetica, Gongronema latifolium* and *Solanum melongena*.

Methods: A single intra-peritoneal (*i.p.*) injection of 90 mg/kg *b.w.* of alloxan monohydrate was given to the rats to induce diabetes mellitus (DM). Serum lipid profiles were measured using standard spectrophotometric methods, whereas atherogenicity, serum lipid ratios and atherogenic coefficient/indices were calculated using standard formulae.

Results: Serum total cholesterol (TC) concentrations of experimental rat groups varied between 1.59 ± 0.10 mmol/L and 2.72 ± 0.16 mmol/L ($p < 0.05$). Serum high-density lipoprotein cholesterol (HDL-C) concentration of untreated DM rat (DM-r) group was significantly lower ($p < 0.05$) than those of treated DM-r groups. Atherogenic risk indices (ARIs) of treated DM-r groups were within the range of 0.74 ± 0.03 and 2.64 ± 0.21, whereas ARI of untreated DM-r was 4.04 ± 0.25. The linear regression analysis of atherognic index of plasma (AIP) versus serum low-density lipoprotein cholesterol (LDL-C) concentrations of the experimental rat groups gave a relatively close fitted regression line ($R^2 = 0.8275$). Atherogenic protection of herbal extract treated DM-r groups was within the range of 33.4–81.7%.

Conclusion: The present study showed that double herbal formulations (DHFs): *A. gangetica* + *G. latifolium* and *A. gangetica* + *A. montanus* offered comparatively high protection to DM-r against atherogenic outcomes, which paralleled the capacities of these DHFs to reverse dyslipidemia.

1. Introduction

Diabetes mellitus (DM) is an endocrine disorder primarily characterized by insufficiency in secretion or action of endogenous insulin, and thereby engenders chronic metabolic syndromes. Etiological classification of DM is based on defects in insulin secretion (Insulin-Dependent DM; IDDM or Type-1-DM) and peripheral tissue resistance to insulin action (Non-Insulin-Dependent DM; NIDDM or Type-2-DM) [1,2]. Additionally, overwhelming level of intracellular oxidative stress in pregnancy may elicit DM; often referred to as gestational DM [3]. Studies have revealed that incidence of Type-1-DM is associated with major environmental factors such as physical inactivity, drugs and toxic agents, obesity and viral infection [4]. Genetic causes have been linked to individuals that are susceptible to Type-2-DM [4]. However, the etiology and progression of DM and its complications are as a result of interplay between genetic and environmental factors [2,4,5].

Epidemiological survey showed that prevalence of DM is rapidly on the rise in developing and developed countries, invigorated by global rising cases of obesity and prevalence of unhealthy lifestyle [2,6]. Specifically, the worldwide 25% adult DM patients' population is expected to double by 2030 [2,7]. Epidemiological survey also reported that cases of DM mortality and morbidity are intertwined with hyperglycemia, dyslipidemia and several distorted metabolic events leading to micro-vascular (retinopathy, nephropathy, neuropathy) and macro-vascular (atherosclerosis and cardiovascular disease) complications [2,8].

Atherogenesis describes the processes in the vascular tissues, facilitated by cellular and acellular elements, which result in the formation of atheromatous plaque on the inner walls of arteries. The formation of atheromatous plaque causes the narrowing of blood flow

Peer review under responsibility of Faculty of Pharmacy, Cairo University.
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https://doi.org/10.1016/j.bfopcu.2018.10.001
Received 21 June 2018; Received in revised form 29 July 2018; Accepted 7 October 2018
Available online 19 October 2018
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channels as well as hardening of the walls of arteries (atherosclerosis) with eventual presentation of cardiovascular diseases (CVD) [7]. CVD-induced coronary and blood vessels lesions are responsible for the pathogenesis of group of disorders; namely, coronary artery disease, cerebrovascular disease, peripheral artery disease, rheumatic heart disease, congenital heart disease, silent myocardial ischemia, deep vein thrombosis, and pulmonary embolism [7,9]. Furthermore, data from empirical investigations revealed that the risk of atherogenic dyslipidemia was raised by two- to three-folds in DM patients [7]. Atherogenic dyslipidemia in DM is associated with increased risk of silent myocardial ischemia and silent coronary artery disease [7,9].

Lipid profile describes proportionate blood lipid composition. Evaluation of blood particulate complex lipid concentrations and constituent ratios offers reliable prognostic and diagnostic data for atherogenic dyslipidemia and ascertaining susceptibility to developing arteriosclerosis as well as predicting cardiovascular morbidity and mortality [10,11]. Elevated serum triacylglycerol (TAG), total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C) and very low-density lipoprotein-cholesterol (VLDL-C) concentrations above their reference intervals are often indications of dyslipidemia and increased incidence of CVD [10]. Elevated serum LDL-C and TAG concentrations are major factors implicated in eliciting higher frequency of cardiovascular events [9,10,12]. Conversely, clinical investigations and empirical data revealed an inverse relationship between serum high-density lipoprotein-cholesterol (HDL-C) concentration and incidence of atherogenic dyslipidemia [9]. The logarithmic ratio of blood TAG concentration to blood HDL-C concentration, the so-called atherogenic index of plasma (AIP), is a reliable predictor of coronary heart disease [9,13,14]. Additionally, atherogenic dyslipidemia is defined in terms of the ratio of serum LDL-C concentration to that of HDL-C concentration [7], referred to as Castelli's risk index II (CRI-II) [10,15]. Ratio of serum [TC]/[HDL-C] denotes Castelli's risk index I (CRI-I) [10,15]. Estimates of CRI-II and CRI-I are useful diagnostic indices for ascertaining level of cardiovascular risk [14,16].

Medicinal plants continue to provide alternative and valuable therapeutic compounds for amelioration of diseases and disorders. The major phyto-constituents of *Acanthus montanus* (Nees) T. Anderson, *Asystasia gangetica* L. T. Anderson, *Gongronema latifolium* Benth and *Solanum melongena* L. Var inerme D.C Hiern have previously been reported by several authors [17–22]. Additionally, the nutraceutical properties of *A. montanus* [23,24], *A. gangetica* [25], *G. latifolium* [17,26–28] and *S. melongena* [29] have previously been reported elsewhere.

We hypothesize that single and combinatorial herbal remedies exert differential capacities to ameliorate atherogenic dyslipidemia and lower atherogenicity risk indicators in DM state. In many traditional healing systems, herbal remedies are usually administered in form of multi-herbal recipe. Accordingly, the present study sought to investigate atherogenicity of alloxan-induced diabetic rats administered single herbal formulations (SHFs), double herbal formulations (DHFs), triple herbal formulations (THFs) and quadruple herbal formulation (QHF) of leaf extracts of *A. montanus*, *A. gangetica, G. latifolium* and *S. melongena*.

2. Materials and methods

2.1. Collection and preparation of samples

Fallow lands in Umuoziri-Inyishi, Ikeduru Local Government Area, Imo State, Nigeria served as sources of fresh leaves of *A. montanus* (ACMO), *A. gangetica* (ASGA). Fresh leaves of *G. latifolium* (GOLA) and *S. melongena* (SOME) were harvested from a botanical garden within the environment of Umuoziri-Inyishi. The leaves were conveyed to the laboratory within 24 h after harvest and identified/authenticated at the Herbarium of the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. The leaves of the selected plants were harvested in wet season during the months of July and August 2016.

The methods previously described by Ojiako et al. [30] were used to prepare the leaves prior to their extraction. The harvested leaves were washed for 15 min using distilled water after which they were allowed to dry at laboratory ambient temperature ($T = 28 \pm 2$ °C). The separate leaves of the selected plants were weighted using a triple beam balance (OHAU 750-50: Burlington, NC, USA) to give 500 g of each leaf sample. The leaves were dried at 60 °C in an oven (WTC BINDER, 7200 Tuttlingen, Germany) to constant weight and until they become crispy. The dried leaves were kept in dark polyethylene bags and stored for 24 h in a cold room at a temperature of 7 ± 3 °C before they were pulverized using Thomas-Willey milling machine (ASTM D-3182, INDIA). Air-tight plastic bottles with screw caps were used to store the ground leaves pending extraction.

2.2. Preparation of leaf extracts

Preparation of ethanolic leaf extracts of the selected plants was according to the methods of Ojiako et al. [30]. The yields were calculated as follows: ACMO = 17.34% (*w/w*), ASGA = 18.12% (*w/w*), GOLA = 19.39% (*w/w*) and SOME = 18.31% (*w/w*). The separate leaf extracts were reconstituted in phosphate buffered saline (PBS), osmotically equivalent to 100 g/L PBS (90.0 g NaCl, 17.0 g $Na_2HPO_4 \cdot 2H_2O$ and 2.43 g $NaH_2PO_4 \cdot 2H_2O$). Corresponding dose of 200 mg/kg body weight (*b.w.*) of single and combinatorial herbal formulations were prepared and administered to the rats. The constituent leaf extracts of the combinatorial herbal formulations were of equal ratios.

2.3. Experimental animals/ethics

One hundred and two (1 0 2) male Wistar rats of weights within the range of 175.35–203.30 g were purchased from a commercial animal house in Owerri-North LGA, Imo State, Nigeria. The rats were kept in well-ventilated metal cages and maintained at room temperature of 28 ± 2 °C, 45–55% of relative humidity on a 12-h light/12-h dark cycle, with access to water and pelletized standard guinea feed (PSGF) (United Africa Company Nigeria Plc., Jos, Nigeria) *ad libitum*. The rats were kept for 2 weeks to acclimatize to environmental conditions. The present study was approved by the Ethical Committee on the use of animals for research, Department of Biochemistry, Federal University Technology, Owerri, Nigeria. Handling of the rats was in accordance with the standard principles of laboratory animal care of the United States National Institutes of Health (NIH, 1978).

2.4. Induction of diabetes mellitus/experimental design

Diabetes mellitus (DM) was induced in the rats by single intraperitoneal (*i.p.*) injection of 90 mg/kg *b.w.* of alloxan monohydrate (Sigma, St. Louis, USA) in PBS (pH = 7.4) [30]. The animals of fasting plasma glucose concentration (FPGC) > 5.71 mmol/L, measured using Accu-Check Sensor Comfort glucometer (Roche, Mexico City), for 5 consecutive days were considered diabetic and selected for the study. A total of 102 male Wistar rats were divided into seventeen (17) groups of six (6) rats each. The animals were deprived of food and water for additional 16 h before commencement of treatment as described elsewhere [30]. The rat groups were designated on the basis of treatments received by oral gavage on daily basis for 21 days as previously described [31]. Leaf extracts used for the treatments of the DM rats (DM-r) were labeled as single herbal formulations (SHFs), double herbal formulations (DHFs), triple herbal formulations (THFs) and quadruple herbal formulation (QHF).

- Group 1 = Normal rats received 1.0 mL/kg *b.w.* of PBS.
- Group 2 = DM-r received 1.0 mL/kg *b.w.* of PBS.
- Group 3 = DM-r received SHF; *A. montanus* (200 mg/kg *b.w.* in PBS; *i.p.*).

- Group 4 = DM-r received SHF; *A. gangetica* (200 mg/kg *b.w.* in PBS; *i.p.*).
- Group 5 = DM-r received SHF; *G. latifolium* (200 mg/kg *b.w.* in PBS; *i.p.*).
- Group 6 = DM-r received SHF; *S. melongena* (200 mg/kg *b.w.* in PBS; *i.p.*)
- Group 7 = DM-r received DHF; (ratio: 1:1 *w/w*) of *A. gangetica* + *A. montanus* (200 mg/kg *b.w.* in PBS; *i.p.*).
- Group 8 = DM-r received DHF; (ratio: 1:1 *w/w*) of *A. gangetica* + *G. latifolium* (200 mg/kg *b.w.* in PBS; *i.p.*).
- Group 9 = DM-r received DHF; (ratio: 1:1 *w/w*) of *A. gangetica* + *S. melongena* (200 mg/kg *b.w.* in PBS; *i.p.*).
- Group 10 = DM-r received DHF; (ratio: 1:1 *w/w*) of *A. montanus* + *G. latifolium* (200 mg/kg *b.w.* in PBS; *i.p.*).
- Group 11 = DM-r received DHF; (ratio: 1:1 *w/w*) of *A. montanus* + *S. melongena* (200 mg/kg *b.w.* in PBS; *i.p.*).
- Group 12 = DM-r received DHF; (ratio: 1:1 *w/w*) of *G. latifolium* + *S. melongena* (200 mg/kg *b.w.* in PBS; *i.p.*).
- Group 13 = DM-r received THF; (ratio: 1:1:1 *w/w*) of *A. gangetica* + *G. latifolium* + *S. melongena* (200 mg/kg *b.w.* in PBS; *i.p.*).
- Group 14 = DM-r received THF; (ratio: 1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *G. latifolium* (200 mg/kg *b.w.* in PBS; *i.p.*).
- Group 15 = DM-r received THF; (ratio: 1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *S. melongena* (200 mg/kg *b.w.* in PBS; *i.p.*).
- Group 16 = DM-r received THF; (ratio: 1:1:1 *w/w*) of *A. montanus* + *G. latifolium* + *S. melongena* (200 mg/kg *b.w.* in PBS; *i.p.*).
- Group 17 = DM-r received QHF; (ratio: 1:1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *G. latifolium* + *S. melongena* (200 mg/kg *b.w.* in PBS; *i.p.*).

2.5. Collection of blood

The 12 h post-fasted rats were sacrificed by cervical dislocation. Blood samples drawn from the orbital sinus of rats were allowed to clot. The serum lipid parameters from corresponding rat groups were measured using standard methods.

2.6. Lipid profile

Serum lipid profile (SLP) was measured on the 21st day of commencement of herbal treatment. Serum TAG, TC and HDL-C concentrations were measured using the Reflovert Plus instrument (Roche, F. Hoffmann-La Roche, Basel, Switzerland) according to the manufacturer's instructions as previously described [5]. Additionally, serum LDL-C concentration was determined according to the formula described by Friedewald et al. [32] as reported by Oluba et al. [33].

$$[\text{LDL - C}] = [\text{TC}] - [\text{HDL - C}] - \left(\frac{[\text{TAG}]}{5}\right) \tag{1}$$

Serum VLDL-C concentration was estimated using the methods of Burnstein and Sammaille [34], where the ratio of serum VLDL-C to TAG concentrations was fixed at 1:5 in fasting animals.

$$[\text{VLDL - C}] = \frac{[\text{TAG}]}{5} \tag{2}$$

2.7. Atherogenic risk index

Atherogenic Risk Index (ARI) was calculated as previously reported [35] thus:

$$\text{ARI} = \frac{[\text{TC} - (\text{HDL - C})]}{[\text{HDL - C}]} \tag{3}$$

2.8. Percentage protection

Percentage protection of herbal extract treated DM-r groups (Groups 3–17) against atherogenicity was calculated thus:

$$\%\,\text{protection} = \frac{\text{ARI}_{\text{Negative control group}} - \text{ARI}_{\text{Treated group}}}{\text{ARI}_{\text{Negative control group}}} \times 100 \tag{4}$$

where:

Negative control group = Untreated DM-r group (Group 2).

Treated group = Herbal extract treated DM-r groups (Groups 3–17).

2.9. Atherogenic risk predictor indices

Atherogenic Risk Predictor Indices (ARPI-1; ARP1-2 and ARPI-3) was calculated as proposed by Dobiasova and Frohlich [36], and previously reported [37] thus:

$$\text{ARPI - 1} = \text{Log}\frac{[\text{TAG}]}{[\text{HDL - C}]} \tag{5}$$

$$\text{ARPI - 2} = \frac{[\text{LDL - C}]}{[\text{HDL - C}]} \tag{6}$$

$$\text{ARP1 - 3} = \frac{[\text{TC}]}{[\text{HDL - C}]} \tag{7}$$

Eq. (5) represents atherognic index of plasma (AIP); [36] Eq. (6) represents Castelli's risk index II (CRI-II); [15] Eq. (7) represents Castelli's risk index I (CRI-I) [15].

2.10. Statistical and data analyses

The data collected was analyzed by the analysis of variance procedure while treatment means was separated by the least significance difference (LSD) incorporated in the statistical analysis system (SAS) package of 9.1 version, (2006). Pearson's regression analysis and correlation coefficient were measured using Excel Software (Microsoft, 2010 version).

3. Results

Table 1 showed that serum TAG concentrations of Groups 1–17 varied between 0.12 ± 0.01 mmol/L and 0.21 ± 0.01 mmol/L ($p < 0.05$). Specifically, Group 1 exhibited the lowest serum TAG concentration, whereas Group 2 gave the highest serum TAG concentration ($p < 0.05$). Serum TAG concentrations of Group 4, Group 9, Group 11, Group 12, Group 16 and Group 17 varied within a relatively narrow range of 0.16 ± 0.01–0.17 ± 0.01 mmol/L ($p > 0.05$). Generally, serum TAG concentration of Group 1 was significantly lower ($p < 0.05$) than those of Groups 3–17. However, serum TAG concentration of Group 2 was significantly higher ($p < 0.05$) than those of Groups 3–17.

Table 1 showed that serum TC concentrations of Groups 1–17 varied between 1.59 ± 0.10 mmol/L and 2.72 ± 0.16 mmol/L ($p < 0.05$). Furthermore, the highest serum TC concentration was exhibited in Group 2, whereas Group 8 gave the lowest serum TC concentration ($p < 0.05$). Specifically, serum TC concentration of Group 8 was 1.7 fold lower than serum TC concentration of Group 2 ($p < 0.05$). Serum TC concentrations showed no significant difference ($p > 0.05$) in Group 1, Group 3, Group 4, Group 5, Group 10, Group 12, Group 14 and Group 15. Serum TC concentrations of Groups 3–17 were significantly ($p < 0.05$) lower than that of Group 2. Likewise, Group 7, Group 8 and Group 11 gave serum TC concentrations that were significantly lower ($p < 0.05$) than that of Group 1.

Table 1
Serum lipid profile of experimental rat groups.

Rat group	Serum lipid profile (mmol/L)				
	TAG	TC	HDL-C	LDL-C	VLDL-C
Group 1	0.12 ± 0.01^{q}	$2.08 \pm 0.11^{f,g,h}$	1.26 ± 0.07^{a}	0.024 ± 0.002^{q}	0.80 ± 0.02^{q}
Group 2	0.21 ± 0.01^{a}	2.72 ± 0.16^{a}	0.54 ± 0.03^{q}	0.041 ± 0.002^{a}	2.14 ± 0.03^{a}
Group 3	0.18 ± 0.01^{b}	$2.20 \pm 0.13^{c,d,e,f}$	$0.75 \pm 0.05^{g,h}$	$0.036 \pm 0.002^{b,c}$	1.41 ± 0.03^{b}
Group 4	$0.17 \pm 0.01^{b,c,d,e}$	$2.08 \pm 0.11^{f,g,h,h,i}$	$0.99 \pm 0.06^{b.c}$	$0.034 \pm 0.002^{e,f}$	$1.06 \pm 0.03^{b,c,d,e}$
Group 5	$0.15 \pm 0.01^{m,n}$	$2.04 \pm 0.10^{f,g,h,I,j,k}$	0.99 ± 0.06^{b}	0.030 ± 0.002^{n}	$1.02 \pm 0.02^{m,n}$
Group 6	$0.18 \pm 0.01^{b,c}$	2.25 ± 0.14^{b}	$0.61 \pm 0.05^{l,m,n,o,p}$	0.036 ± 0.002^{b}	$1.60 \pm 0.03^{b,c}$
Group 7	$0.14 \pm 0.01^{n,o}$	$1.73 \pm 0.10^{o,p}$	$0.95 \pm 0.05^{b,c,d}$	0.028 ± 0.002^{o}	$0.50 \pm 0.02^{n,o}$
Group 8	$0.14 \pm 0.01^{n,o,p}$	$1.59 \pm 0.10^{p,q}$	$0.91 \pm 0.05^{b,c,d,e,f}$	$0.028 \pm 0.002^{o,p}$	$0.65 \pm 0.02^{n,o,p}$
Group 9	$0.17 \pm 0.01^{b,c,d,e,f}$	$2.31 \pm 0.13^{b,c,d}$	0.64 ± 0.05^{l}	0.034 ± 0.002^{e}	$1.64 \pm 0.03^{b,c,d,e,f}$
Group 10	$0.16 \pm 0.01^{d,e,f,g,h,I,j,k,l,m}$	$2.03 \pm 0.10^{f,g,h,I,j,k,l}$	$0.92 \pm 0.05^{b,c,d,e}$	$0.032 \pm 0.002^{h,I,j,k,l,m}$	$1.08 \pm 0.03^{d,e,f,g,h,I,j,k,l,m}$
Group 11	$0.16 \pm 0.01^{d,e,f,g,h,I,j}$	1.79 ± 0.10^{o}	$0.74 \pm 0.05^{g,h,i,j}$	$0.032 \pm 0.002^{h,I,j,k,l}$	$1.02 \pm 0.03^{d,e,f,g,h,I,j}$
Group 12	$0.16 \pm 0.01^{d,e,f.g.h}$	$2.30 \pm 0.13^{b,c,d,e,f}$	0.78 ± 0.05^{g}	$0.032 \pm 0.002^{h,I,j,k}$	$1.49 \pm 0.03^{d,e,f.g.h}$
Group 13	$0.18 \pm 0.01^{b,c,d}$	$2.32 \pm 0.13^{b,c}$	$0.64 \pm 0.05^{l,m,n}$	$0.036 \pm 0.02^{b,c,d}$	$1.64 \pm 0.03^{b,c,d}$
Group 14	$0.16 \pm 0.01^{d,e,f,g,h,I,j,k,l}$	$2.04 \pm 0.10^{f,g.h.i.j}$	$0.61 \pm 0.04^{l,m,n,o}$	$0.032 \pm 0.02^{h,i,j}$	$1.40 \pm 0.03^{d,e,f,g,h,I,j,k,l}$
Group 15	$0.16 \pm 0.01^{d,e,f,g,h,I,j,k}$	$2.16 \pm 0.13^{c,d,e,f,g}$	$0.69 \pm 0.05^{l,m}$	$0.032 \pm 0.02^{h,i}$	$1.44 \pm 0.03^{d,e,f,g,h,I,j,k}$
Group 16	$0.17 \pm 0.01^{d,e,f,g}$	$1.98 \pm 0.10^{g,h,I,j,k,l,m,n}$	$0.72 \pm 0.05^{g,h,I,j,k}$	$0.034 \pm 0.02^{e,f,g}$	$1.23 \pm 0.03^{d,e,f,g}$
Group 17	$0.16 \pm 0.01^{d,e,f,g,h,i}$	$2.01 \pm 0.10^{g,h,I,j,k,l,m}$	$0.74 \pm 0.05^{g,h,i}$	0.032 ± 0.02^{h}	$1.24 \pm 0.03^{d,e,f,g,h,i}$

The mean (X) ± S.D of six ($n = 6$) determinations. Means in the column with the same letter are not significantly different at $p > 0.05$ according to LSD.

Serum HDL-C concentration of Group 1 was 2.33 folds higher than that of Group 2 ($p < 0.05$) (Table 1). Serum HDL-C concentration of Group 2 was significantly lower ($p < 0.05$) than those of Groups 3–17. Comparatively, Group 4, Group 5, Group 7, Group 8 and Group 10 gave serum HDL-C concentrations of no significant difference ($p > 0.05$). Likewise, serum HDL-C concentrations of Group 3, Group 11, Group 12, Group 16 and Group 17 showed no significant difference ($p > 0.05$). Group 6, Group 9, Group 13, Group 14 and Group 15 gave relatively low serum HDL-C concentrations. Table 1 showed that serum LDL-C concentration of Groups 1–17 was within the range of 0.024 ± 0.002 mmol/L and 0.041 ± 0.02 mmol/L ($p < 0.05$). Group 2 gave the highest serum LDL-C concentration, whereas that of Group 8 exhibited the lowest serum LDL-C concentration. Serum LDL-C concentration of Group 2 represented 1.46 folds increase in serum LDL-C concentration compared with that of Group 8 ($p > 0.05$).

Serum LDL-C concentrations of Groups 3–17 were significantly higher ($p < 0.05$) than that of Group 1. Conversely, serum LDL-C concentrations of Groups 3–17 were significantly lower ($p < 0.05$) than that of Group 2. Table 1 showed that serum LDL-C concentrations of Group 12, Group 14 and Group 15 exhibited no significant different ($p > 0.05$). Serum VLDL-C concentrations were 5 folds lower than serum TAG concentrations of corresponding rat groups (Table 1).

The ARI and ARPIs are presented in Table 2. Specifically, Table 2 showed that Group 2 exhibited the highest ARI, ARPI-1 and ARPI-3 compared with other experimental rat groups.

The ARIs of Groups 3–17 were significantly ($p < 0.05$) lower than that of Group 2. Specifically, ARIs of Groups 3–17 were within the range of 0.74 ± 0.03 and 2.64 ± 0.21, whereas ARI of Group 2 was 4.04 ± 0.25. However, ARIs of Groups 3–17 were significantly higher ($p < 0.05$) than that of Group 1; except that of Group 8 ($p > 0.05$). Additionally, ARI of Group 17 was not significantly different ($p < 0.05$) from that of Group 16. An overview of Table 2 showed that ARIs of Group 3, Group 12 and Group 15 exhibited no significant difference ($p > 0.05$).

Table 2 showed that logarithm of ARI of Group 1, Group 7 and Group 8 gave negative values, whereas logarithm of ARI of Group 2 gave the highest positive value. Furthermore, positive values of logarithm of ARI of the treated rat groups following herbal treatments were within the range of 0.43 ± 0.02, which corresponded to that of Group 6, and 0.03 ± 0.001, which corresponded to that of Group 5.

The linear regression analysis of AIP versus serum LDL-C concentrations of the experimental rat groups (Groups 1–17) gave a relatively close fitted regression line ($R^2 = 0.8275$) (Fig. 1). Additionally, Pearson's correlation analysis of AIP versus serum LDL-C concentrations of Groups 1–17 gave a strong positive correlation ($r = 0.909659725$).

Atherogenic protection of herbal extract treated DM-r groups (Group 3–17) was within the range of 33.4–81.7% (Table 3). Additionally, the results in Table 3 showed that Group 8 exhibited the highest atherogenic protection, whereas that of Group 6 was the lowest. Generally, Group 3, Group 6, Group 9, Group 13, Group 14 and Group 15 exhibited atherogenic protections that were below 50%.

4. Discussion

The present study revealed evidence of dyslipidemia in alloxan-induced DM rats, which was in conformity with previous reports [5,38,39]. Evidence from animal models also showed the potency of herbal extracts to reverse diabetic dyslipidemia [5,38,39]. Additionally, epidemiological investigations showed that consumption of food materials rich in phytochemical antioxidants like polyphenolic compounds offered lower risk of DM pathogenesis as well as its complications and predisposing factors [40]. Previous empirical investigations showed that combinations of saponins from *Glycyrrhiza glabra* (F. Fabaceae), *Withania somnifera* (F. Solanaceae), *Asparagus racemosus* (F. Liliaceae), *Chlorophytum borivilianum* (F. Liliaceae) and *Sesamum indicum* (F. Pedaliaceae) ameliorated dyslipidemia in hypercholesterolemic rats by improving fecal excretion of cholesterol, neutral sterol and bile acids [41]. Likewise, saponins from fruit extracts of *Solanum anguivi* ameliorated dyslipidemia in alloxan-induced DM-r [38]. According to the findings of Bhavsar et al. [42] saponins ameliorated dyslipidemia and related metabolic syndromes by promoting increased gene expression responsible for biosynthesis of glucose transporter-4 (GLUT-4), adipsin and peroxisome proliferator-activated receptor gamma (PPARγ), while simultaneously, caused decreased gene expression of fatty acid binding protein 4 (FABP4) and glucose-6-phosphatase. Likewise, hypercholesterolemia in streptozotocin-induced DM rats were ameliorated following the administration of tannin supplements from *Ficus racemosa* [43]. Furthermore, the roles of alkaloids and flavonoids to reverse dyslipidemia in animal models have previously been reported elsewhere [5]. Based on these previous findings, the present study therefore suggests that the relatively high content of saponins, alkaloids, flavonoids and tannins in DHFs of *A. gangetica* + *A. montanus* and *A. gangetica* + *G. latifolium* appeared to have contributed to the capacities the DHFs to reverse dyslipidemia in DM-r.

Clinical investigations have revealed that individuals that exhibit high ARI as well as atherogenicity risk predictor indices and biomarkers

Table 2
Atherogenicity and atherogenic risk predictor indices of experimental rat groups.

Parameters/Rat group	$\frac{[TC - (HDL - C)]}{HDL - C}$	$Log\frac{[TC - (HDL - C)]}{HDL - C}$	$Log\frac{[TAG]}{HDL - C}$	$\frac{[LDL - C]}{[HDL - C]}$	$\frac{[TC]}{[HDL - C]}$
Group 1	0.65 ± 0.02[q]	−0.19 ± 0.01	−1.02 ± 0.01	0.02 ± 0.001	1.64 ± 0.02
Group 2	4.04 ± 0.25[a]	0.61 ± 0.02	−0.41 ± 0.02	0.08 ± 0.001	5.00 ± 0.11
Group 3	2.07 ± 0.11[e,f,g,h]	0.32 ± 0.01	−0.62 ± 0.01	0.05 ± 0.001	2.94 ± 0.11
Group 4	1.10 ± 0.09[l,m]	0.04 ± 0.001	−0.77 ± 0.01	0.03 ± 0.001	2.08 ± 0.11
Group 5	1.06 ± 0.08[m,n]	0.03 ± 0.001	−0.82 ± 0.01	0.03 ± 0.001	2.04 ± 0.11
Group 6	2.69 ± 0.21[b]	0.43 ± 0.02	−0.53 ± 0.01	0.06 ± 0.001	3.70 ± 0.11
Group 7	0.82 ± 0.03[o]	−0.08 ± 0.001	−0.83 ± 0.01	0.03 ± 0.001	1.82 ± 0.02
Group 8	0.74 ± 0.03[o,p]	−0.13 ± 0.01	−0.81 ± 0.01	0.03 ± 0.001	1.75 ± 0.04
Group 9	2.61 ± 0.19[c,d]	0.42 ± 0.02	−0.58 ± 0.02	0.05 ± 0.001	3.57 ± 0.11
Group 10	1.21 ± 0.11[l]	0.08 ± 0.001	−0.76 ± 0.01	0.03 ± 0.001	2.22 ± 0.11
Group 11	1.42 ± 0.17[k]	0.15 ± 0.01	−0.67 ± 0.01	0.04 ± 0.001	2.44 ± 0.11
Group 12	1.95 ± 0.13[f,g]	0.29 ± 0.01	−0.69 ± 0.01	0.04 ± 0.001	2.94 ± 0.11
Group 13	2.63 ± 0.23[c]	0.42 ± 0.02	−0.55 ± 0.02	0.06 ± 0.001	3.57 ± 0.11
Group 14	2.34 ± 0.21[e]	0.37 ± 0.01	−0.58 ± 0.01	0.05 ± 0.001	3.33 ± 0.11
Group 15	2.13 ± 0.20[e,f]	0.33 ± 0.01	−0.63 ± 0.01	0.05 ± 0.001	3.13 ± 0.11
Group 16	1.76 ± 0.10[h,I,j]	0.25 ± 0.01	−0.63 ± 0.01	0.05 ± 0.001	2.78 ± 0.11
Group 17	1.72 ± 0.11[h,i]	0.24 ± 0.01	−0.67 ± 0.01	0.04 ± 0.001	2.70 ± 0.12

The mean (X) ± S.D of six (n = 6) determinations. Means in the column with the same letter are not significantly different at $p > 0.05$ according to LSD.
$ARI = \frac{[TC - (HDL - C)]}{[HDL - C]}$.
$ARPI - 1 = Log\frac{[TAG]}{[HDL - C]}$; $ARPI - 2 = \frac{[LDL - C]}{[HDL - C]}$; $ARP1 - 3 = \frac{[TC]}{[HDL - C]}$.
[LDL-C]/[HDL-C] ratio > 2.3 is atherogenic and undesirable; [TC]/[HDL-C] ratio > 3.33 is atherogenic and undesirable.

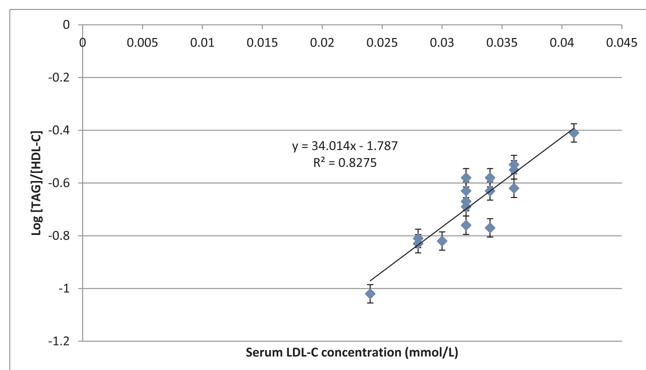

Fig. 1. Linear regression analysis of AIP versus serum LDL-C concentrations of experimental rat groups.

Table 3
Percentage protection of herbal extract treated rat groups.

Rat group	Herbal formulations administered (200 mg/kg *b.w.* in PBS)	% protection
Group 3	SHF; *A. montanus*	48.8
Group 4	SHF; *A. gangetica*	72.8
Group 5	SHF; *G. latifolium*	73.8
Group 6	SHF; *S. melongena*	33.4
Group 7	DHF; *A. gangetica* + *A. montanus*	79.7
Group 8	DHF; *A. gangetica* + *G. latifolium*	81.7
Group 9	DHF; *A. gangetica* + *S. melongena*	35.4
Group 10	DHF; *A. montanus* + *G. latifolium*	70.0
Group 11	DHF; *A. montanus* + *S. melongena*	64.9
Group 12	DHF; *G. latifolium* + *S. melongena*	51.7
Group 13	THF; *A. gangetica* + *G. latifolium* + *S. melongena*	34.9
Group 14	THF; *A. montanus* + *A. gangetica* + *G. latifolium*	42.1
Group 15	THF; *A. montanus* + *A. gangetica* + *S. melongena*	47.3
Group 16	THF; *A. montanus* + *G. latifolium* + *S. melongena*	56.4
Group 17	QHF; *A. montanus* + *A. gangetica* + *G. latifolium* + *S. melongena*	57.4

such as AIP, CRI-I and CRI-II run high risk of developing atherosclerosis and coronary heart disease [10,13–15]. Studies have shown that serum lipid ratios and atherogenic coefficient/indices are better predictor of CVD than isolated serum lipid parameters [11]. Mathematically, relatively low ARI is achieved by lowering of plasma concentrations of atherogenic lipoproteins, particularly LDL-C as well as esterified and non-esterified plasma lipids such as TAG and TC with corresponding elevation of plasma concentration of HDL-C. The strong positive correlation (r = 0.909659725) between AIP and serum LDL-C concentrations of Groups 1–17 was an obvious indication that elevated serum LDL-C concentration engendered corresponding raised AIP value and associated risk indicators. Additionally, the relatively close fitted regression line (R^2 = 0.8275) between AIP and serum LDL-C concentrations of Groups 1–17 was an indication that serum LDL-C was a determinant and predictor of AIP risk indicators, and therefore, raised serum LDL-C concentration contributed to atherogencity in DM. Accordingly, therapeutic and dietary interventions cause re-adjustments of atherogenic indicators to the advantage of the individual and animal models. For the most part, therapeutic and dietary interventions are specifically targeted to lower levels of atherogenic plasma lipoproteins as well as inhibit the biosynthesis but enhanced elimination of plasma cholesterol [7,44].

5. Conclusion

The present study showed that DHFs: *A. gangetica* + *G. latifolium* and *A. gangetica* + *A. montanus* offered comparatively high protection to DM-r against atherogenic outcomes, which paralleled the capacities of these DHFs to reverse dyslipidemia. Finally, the present findings appeared to suggest synergy among specific bioactive principles from DHFs of *A. gangetica* + *G. latifolium* and *A. gangetica* + *A. montanus*, which potentiated their capacities to ameliorate atherogenic dyslipidemia as previously discussed. Further studies using bioassay-guided approach is required to identify the bioactive principles that ameliorated atherogenic dyslipidemia in DM-r, in order to isolate and characterize these therapeutic biomolecules as well as elucidate their mechanisms of therapeutic action.

6. Financial support and sponsorship

Nil.

7. Conflict of interest statement

The authors declare that there is no conflict of interest regarding the

publication of this article.

8. Authors' contributions

OAO conceived and designed the research and supervised the laboratory work. PCC prepared the manuscript and analyzed the data. CMC collected the plant samples and carried out the laboratory work. AAE analyzed the data and supervised the laboratory work. All authors read and approved the final manuscript.

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