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Original article

Blood glucose level and lipid profile of alloxan-induced hyperglycemic rats treated with single and combinatorial herbal formulations

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ABSTRACT

The current study sought to investigate the capacities of single and combinatorial herbal formulations of leaf extracts of *Acanthus montanus*, *Asystasia gangetica*, *Emilia coccinea*, and *Hibiscus rosasinensis* to reverse hyperglycemia and dyslipidemia in alloxan-induced diabetic male rats. Phytochemical composition of the herbal extracts, fasting plasma glucose concentration (FPGC), and serum lipid profile (SLP) of the rats were measured by standard methods. The relative abundance of phytochemicals in the four experimental leaf extracts was in the following order: flavonoids > alkaloids > saponins > tannins. Hyperglycemic rats (HyGR) treated with single and combinatorial herbal formulations showed evidence of reduced FPGC compared with the untreated HyGR and were normoglycemic (FPGC < 110.0 mg/dL). Similarly, HyGR treated with single and combinatorial herbal formulations showed evidence of readjustments in their SLPs. Generally, HyGR treated with triple herbal formulations (THfs) exhibited the highest atherogenic index compared with HyGR treated with single herbal formulations (SHfs), double herbal formulations (DHfs), and quadruple herbal formulation (QHf). The display of synergy or antagonism by the composite herbal extracts in ameliorating hyperglycemia and dyslipidemia depended on the type and number of individual herbal extract used in constituting the experimental herbal formulations. Furthermore, the capacities of the herbal formulations (SHfs, DHfs, THfs, and QHf) to exert glycemic control and reverse dyslipidemia did not follow predictable patterns in the animal models.

1. Introduction

Hyperglycemia and dyslipidemia, among other disorders, are metabolic syndromes associated with a dysfunctional endocrine system clinically referred to as diabetes mellitus (DM).[1–3] DM is described and classified on the basis of intrinsic and extrinsic causative factors, which has been exhaustively explained elsewhere.[4–7] Although the etiology of DM is multifaceted, the prevalence of the disease worldwide is often linked to genetic/physiologic factors, sedentary lifestyle, and obesity,[8–11] of which poor dietary habits such as high consumption of sugars and saturated fats in addition to low intake of polyunsaturated fatty acids (PUFAs) have been implicated to be major contributory factors toward the progression of the disease.[12,13]

The earliest and common diagnostic indices of DM are hyperglycemia and glucosuria. In that regard, the unusual metabolism of carbohydrates in DM, and associated profound adjustments of glycolytic pathways[14,15] engender the activation of alternative polyol metabolic pathways with resultant intracellular accumulation of sorbitol[16] and auto-oxidation of glucose.[17] These distortional metabolic events have been implicated in the etiology of diabetic peripheral neuropathy, retinopathy, and cataracts.[9,18] Patterns of dyslipidemia in DM and connecting primary risk factors have been described in earlier reports.[2,19,20] Atherosclerosis-induced coronary heart disease (CHD), stroke, and hypertension are major causes of increasing rate of fatalities among patients with DM.[21,22]

The dilapidating action of DM qualifies it as a disease of major public health concern and epidemiological survey showed that it is the seventh leading cause of death worldwide.[23] Additionally, projections showed that the disease will become the foremost

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cause of morbidity and mortality within the next 25 years, especially in Africa and Asia.[5,9,24] In addition to the option of DM management that involves intramuscular administration of insulin to individuals with diabetes, there are several synthetic antidiabetic medicinal preparations of notable capacity to act as agents of glycemic control.[25–27] However, from a toxicological standpoint, alternative herbal formulation remedies are sometimes preferred to synthetic antidiabetic drugs because of its minimal or no side effects.[28,29] Furthermore, because the uses of traditional plant medicines are cost-effective mitigation strategies, ethnomedicinal practices are being increasingly applied worldwide for the alleviation and management of DM.[9,18,24,30–33] Decoctions from parts or whole plants of *Acanthus montanus*, *Asystasia gangetica*, *Emilia coccinea*, and *Hibiscus rosasinensis* have been effectively applied for the treatment and management of numerous pathologic conditions.[34–39] Most ethnomedicinal practitioners presume that administration of combinatorial extracts of different plant species serves to potentiate the efficacy of herbal concoctions[40] and may exhibit competitive therapeutic potentials when compared with that of orthodox medicines.[41] Accordingly, the current study sought to investigate the capacities of single and combinatorial herbal formulations of leaf extracts of *A. montanus*, *A. gangetica*, *E. coccinea*, and *H. rosasinensis* to reverse hyperglycemia and dyslipidemia in alloxan induced diabetic male rats.

2. Materials and methods

2.1. Collection and preparation of herbal samples

Fresh leaves of *Acanthus montanus* (Nees) T. Anderson (ACMO), *Emilia coccinea* G. Don (EMCO), and *Hibiscus rosasinensis* L. (HIRO) were collected from uncultivated lands in Umuamacha Ayaba Umaeze, Osisioma Ngwa Local Government Area (LGA), Abia State, Nigeria, whereas fresh leaves of *Asystasia gangetica* L. T. Anderson (ASGA) were collected from Ubowuala, Emekuku, Owerri North Local Government Area (LGA), Imo State, Nigeria. The four herbs were identified and authenticated by Dr. M. Ibe, School of Agriculture and Agricultural Technology (SAAT), Federal University of Technology, Owerri, Nigeria. All the leaves were collected between the months of July 2009 and August 2009.

The leaves of individual plants were washed with continuous flow of distilled water for 15 minutes and allowed to dry at laboratory ambient temperature (24 ± 5 °C). A 500 g portion of each of the herbal samples were weighted using a triple beam balance (OHAU 750-50: Burlington, NC, USA) and dried in an oven (WTC BINDER, 7200 Tuttlingen, Germany) at 60 °C until a constant weight was achieved. The dried leaves were packaged in dark polyethylene bags and kept in cold room (7 ± 3 °C) for 24 hours prior to pulverization. Next, the separate dried leaves were pulverized using a Thomas-Willey milling machine (Thomas Wiley® Mini-Mill; ASTM D-3182; India), after which the ground samples were stored in air-tight plastic bottles with screw caps pending extraction.

2.2. Extraction of herbal samples

A 40 g portion of each **of the** pulverized dried samples of *A. montanus*, *A. gangetica*, *E. coccinea*, and *H. rosasinensis* were subjected to repeated Soxhlet extraction cycles for 2 hours using 96% C_2H_5OH (BDH, UK) as solvent to obtain a final volume of 500 mL of each herbal extract. These volumes of the herbal extracts were concentrated and recovered in a rotary evaporator for 12 hours at 60 °C under reduced pressure. The extracts were dried in a desiccator for 24 hours, wrapped in aluminum foil, and stored in air-tight plastic bottles with screw caps at ≤ 4 °C. The yields were calculated to be as follows: *A. montanus* = 16.35% (*w/w*), *A. gangetica* = 16.69% (*w/w*), *E. coccinea* = 17.99% (*w/w*), and *H. rosasinensis* = 17.23% (*w/w*). The separate extracts were reconstituted in phosphate buffered saline (PBS) solution (extract vehicle), osmotically equivalent to 100 g/L PBS (90.0 g NaCl, 17.0 g $Na_2HPO_4.2H_2O$, and 2.43 g $NaH_2PO_4.2H_2O$), before appropriate doses were administered to the experimental animals. Portions of the individual herbal extracts were also measured for their phytochemical contents.

2.3. Phytochemical composition of herbal extracts

Flavonoids content was measured by the methods of Boham and Kocipai.[42] The concentration of alkaloids of the herbal extracts was measured by the methods of Harborne.[43] Measurement of saponin content of the herbal extracts was performed according to the methods of Harborne,[43] as reported by Obadoni and Ochuko.[44] The Van-Burden and Robinson[45] method as reported by Belonwu et al[46] was used to measure concentration of tannins of the herbal extracts.

2.4. Experimental animals

Male albino (Wistar) rats (School of Agriculture and Agricultural Technology (SAAT), Federal University of Technology, Owerri, Nigeria) weighing between 150–160 g were maintained at room temperatures of 24 ± 5 °C, 30–55% of relative humidity on a 12-hour light/12-hour dark cycle, with access to water and standard commercial feed (SCF; Ewu Feed Mill, Edo State, Nigeria) *ad libitum* for a 2-week acclimatization period. The handling of the animals was in accordance with the standard principles of laboratory animal care of the United States National Institutes of Health (NIH, 1978).

2.5. Induction of diabetes/experimental design

Hyperglycemia was induced in the experimental rats by single intraperitoneal (i.p.) injection of 90 mg/kg body weight of alloxan monohydrate (Sigma-Aldrich, St. Louis, MO, USA) in PBS solution (pH = 7.4). The animals with fasting plasma glucose concentration (FPGC) > 110 mg/dL for 5 consecutive days were considered hyperglycemic and selected for the study. A total of 102 male Wistar rats were allotted into 17 groups of six rats each. The animals were deprived of food and water for an additional 16 hours prior to the commencement of treatment as described elsewhere.[47] The animal groups were designated on the basis of treatments received at regular intervals of 2 days for 30 days. Herbal treatments of the hyperglycemic rats (HyGR) were described as single herbal formulations (SHf): (HrACMO, HrASGA, HrEMCO, and HrHIRO), double herbal formulations (DHf): (HrAGAM, HrAGEC, HrAGHR, HrAMEC, HrAMHR, and HrECHR), triple herbal formulations (THf): (HrAGEH, HrAMAE, HrAMAH, and HrAMEH), and quadruple herbal formulation (QHf): (HrAAEH).

- NORM: Normal rats received SCF + water *ad libitum* + 1.0 mL/kg of PBS.
- DIAB: HyGR received SCF + water *ad libitum* + 1.0 mL/kg of PBS.
- HrACMO: HyGR received SCF + water *ad libitum* + *A. montanus* (20 mg/kg in PBS; i.p.).
- HrASGA: HyGR received SCF + water *ad libitum* + *A. gangetica* (20 mg/kg in PBS; i.p.).
- HrEMCO: HyGR received SCF + water *ad libitum* + *E. coccinea* (20 mg/kg in PBS; i.p.).
- HrHIRO: HyGR received SCF + water *ad libitum* + *H. rosasinensis* (20 mg/kg in PBS; i.p.)
- HrAGAM: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *A. montanus* (20 mg/kg in PBS; i.p.).

- HrAGEC: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *E. coccinea* (20 mg/kg in PBS; i.p.).
- HrAGHR: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *H. rosasinensis* (20 mg/kg in PBS; i.p.).
- HrAMEC: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1 *w/w*) of *A. montanus* + *E. coccinea* (20 mg/kg in PBS; i.p.).
- HrAMHR: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1 *w/w*) of *A. montanus* + *H. rosasinensis* (20 mg/kg in PBS; i.p.).
- HrECHR: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1 *w/w*) of *E. coccinea* + *H. rosasinensis* (20 mg/kg in PBS; i.p.).
- HrAGEH: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1:1 *w/w*) of *A. gangetica* + *E. coccinea* + *H. rosasinensis* (20 mg/kg in PBS; i.p.).
- HrAMAE: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *E. coccinea* (20 mg/kg in PBS; i.p.).
- HrAMAH: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *H. rosasinensis* (20 mg/kg in PBS; i.p.).
- HrAMEH: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *E. coccinea* + *H. rosasinensis* (20 mg/kg in PBS; i.p.).
- HrAAEH: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *E. coccinea* + *H. rosasinensis* (20 mg/kg in PBS; i.p.).

Blood volumes of 2.0 mL were drawn from 12-hour post-fasted animals on the 30th day experimental period and measured for fasting plasma glucose concentration (FPGC) and serum lipid profile (SLP).

2.6. Fasting plasma glucose concentration

Blood samples were drawn from the orbital sinus[48] and measured for FPGC. Estimation of FPGC was by the glucose oxidase method according to Randox kit manufacturer's procedure (Randox Laboratories Ltd, Ardmore, UK).

2.7. Serum lipid profile

Blood samples were obtained from the various experimental animal groups and measured for SLP according to the methods previously described.[49] Total cholesterol (TC), triacylglycerol (TAG), and high-density lipoprotein cholesterol (HDL-C) were measured using commercial kits (Randox Laboratory Ltd, Crumlin, Co. Antrim, UK). Low-density lipoprotein cholesterol (LDL-C) concentration was estimated according to the formula of Friedewald et al[50];

$$\text{LDL-C} = \text{TC} - (\text{HDL-C}) - (\text{TAG}/5), \quad (1)$$

as reported by Shaker et al.[51] The atherogenic index (AI) was calculated as follows:

$$[\text{TC-(HDL-C)}]/(\text{HDL-C}).[52] \quad (2)$$

2.8. Statistical analysis

The results were expressed as mean ± standard error of the mean, and statistically analyzed by one-way analysis of variance followed by the Dunnett test, with the level of significance set at $p < 0.05$.

3. Results

Table 1 shows that flavonoids were, in comparison, the most abundant phytochemical present in the four herbal extracts, which was in the following order: AMCO > ASGA > HIRO > EMCO; $p < 0.05$. The concentration of alkaloids in HIRO was relatively low compared with the other three herbal extracts, whereas EMCO gave the highest concentration of alkaloids. Saponins contents of the four herbal extracts were in the range of 43.50 ± 0.54 mg/g and 71.01 ± 0.62 mg/g. In addition, saponins contents amongst ASGA, EMCO, and HIRO herbal extracts were not significantly different ($p > 0.05$) but significantly ($p < 0.05$) lower than saponins content of ACMO. Specifically, saponins contents were as follows: ASGA = 44.50 ± 0.51 mg/g, EMCO = 43.50 ± 0.54 mg/g, and HIRO = 45.25 ± 0.35 mg/g (Table 1). Likewise, tannin contents of the four herbal extracts was within a narrow range of 25.50 ± 0.18 mg/g and 33.75 ± 0.32 mg/g; $p > 0.05$. In addition, among the four phytochemicals measured, tannin was the lowest phytochemical present in the four herbal extracts.

The results presented in Fig. 1 show that FPGC of untreated HyGR (DIAB group) was 4.26-fold higher than that of the control/normal rats (NORM group). HyGR treated with SHf exhibited relatively lower FPGC compared with the DIAB group. However, HrACMO, HrEMCO, and HrHIRO showed evidence of hyperglycemia (FPGC > 110 mg/dL) except $\text{HrASGA}_{[\text{FPGC}]}$ = 105.7 ± 0.71 mg/dL. Similarly, HyGR treated with DHf showed evidence of relatively reduced FPGC compared with the DIAB group and were normoglycemic (FPGC < 110.0 mg/dL); except $\text{HrAGHR}_{[\text{FPGC}]}$ = 112.5 ± 0.42 mg/dL. Specifically, $\text{HrAGEC}_{[\text{FPGC}]}$ = 81.0 ± 0.39 mg/dL and $\text{HrAGAM}_{[\text{FPGC}]}$ = 66.3 ± 0.71 mg/dL were normoglycemic compared with $\text{NORM}_{[\text{FPGC}]}$ = 86.3 ± 0.51 mg/dL. HyGR treated with THf: $\text{HrAMAE}_{[\text{FPGC}]}$ = 84.7 ± 0.51 mg/dL was normoglycemic, whereas $\text{HrAMAH}_{[\text{FPGC}]}$ = 124.3 ± 0.92 mg/dL indicated persistent hyperglycemia after the 30-day treatment.

Serum total cholesterol (TC) of the DIAB group was not significantly different ($p > 0.05$) from that of the NORM group (Fig. 2). HyGR treated with SHf; $\text{HrACMO}_{[\text{TC}]}$ = 44.17 ± 2.31 mg/dL and $\text{HrASGA}_{[\text{TC}]}$ = 45.0 ± 2.92 mg/mL, exhibited reduced serum TC that were significantly ($p < 0.05$) lower than that of the NORM group. However, $\text{HrEMCO}_{[\text{TC}]}$ = 61.2 ± 2.13 mg/dL and $\text{HrHIRO}_{[\text{TC}]}$ = 58.0 ± 2.43 mg/dL were comparable with serum TC of the NORM group; $p > 0.05$. Serum TC of HyGR treated with DHf was within the range of 34.7 ± 2.34 mg/dL – 55.8 ± 1.53 mg/dL. Serum TC of HrAGAM, HrAGEC, HrAGHR, HrAMEC, and HrAMHR showed no significant difference ($p > 0.05$) and were lower than the MORM group. $\text{HrECHR}_{[\text{TC}]}$ = 55.8 ± 1.53 mg/dL was not significantly different ($p > 0.05$) from serum TC of HyGR treated with SHf. Serum

Table 1
Some phytochemical contents of *Acanthus montanus*, *Asystasia gangetica*, *Emilia coccinea*, and *Hibiscus rosasinensis* herbal extracts.

Sample	Concentrations of phytochemicals (mg/g dry sample)			
	Alkaloids	Flavonoids	Saponins	Tannins
ACMO	$177.25 \pm 1.56^{b,c}$	561.00 ± 2.11^{a}	71.00 ± 0.62^{a}	$26.5 \pm 0.23^{a,b,c}$
ASGA	188.25 ± 1.02^{b}	450.50 ± 1.98^{b}	$44.50 \pm 0.51^{b,c}$	33.75 ± 0.32^{a}
EMCO	352.75 ± 0.95^{a}	345.00 ± 0.89^{d}	$43.50 \pm 0.54^{b,c,d}$	$29.50 \pm 0.29^{a,b}$
HIRO	70.00 ± 0.67^{d}	$425.00 \pm 1.71^{b,c}$	45.25 ± 0.35^{b}	$25.50 \pm 0.18^{a,b,c,d}$

The mean (X) ± standard deviation of six ($n = 6$) determinations. Means in the column with the same letter are not significantly different at $p > 0.05$.
ACMO = *Acanthus montanus*; ASGA = *Asystasia gangetica*; EMCO = *Emilia coccinea*; HIRO = *Hibiscus rosasinensis*.

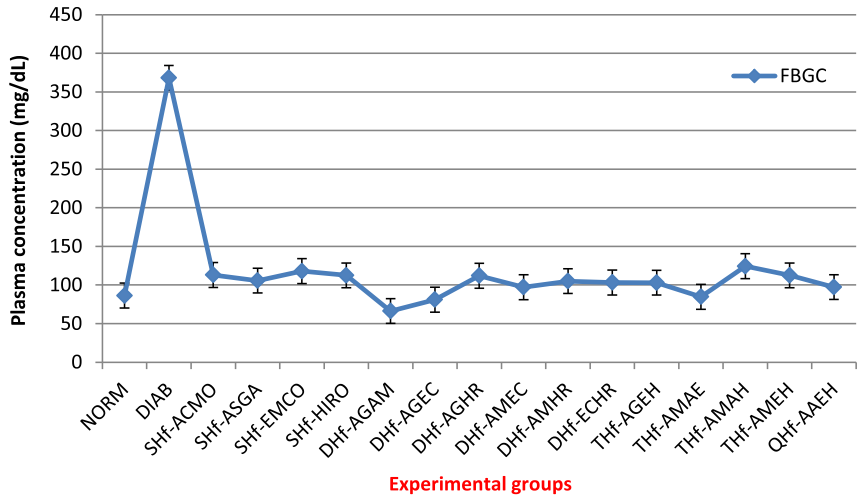

Fig. 1. Fasting plasma glucose concentrations of normal, diabetic, and treated rats. ACMO = *Acanthus montanus*; ASGA = *Asystasia gangetica*; DHf = double herbal formula; EMCO = *Emilia coccinea*; HIRO = *Hibiscus rosasinensis*; NORM = normal; SHf = single herbal formula; THf = triple herbal formula. AGAM = combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *A. montanus*; AGEC = combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *E. coccinea*; AGHR = combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *H. rosasinensis*; AMEC = combined dose (ratio: 1:1 *w/w*) of *A. montanus* + *E. coccinea*; AMHR = combined dose (ratio: 1:1 *w/w*) of *A. montanus* + *H. rosasinensis*; ECHR = combined dose (ratio: 1:1 *w/w*) of *E. coccinea* + *H. rosasinensis*; AGEH = combined dose (ratio: 1:1:1 *w/w*) of *A. gangetica* + *E. coccinea* + *H. rosasinensis*; AMAE = combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *E. coccinea*; AMAH = combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *H. rosasinensis*; AMEH = combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *E. coccinea* + *H. rosasinensis*; AAEH = combined dose (ratio: 1:1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *E. coccinea* + *H. rosasinensis*; FPGC = fasting plasma glucose concentration.

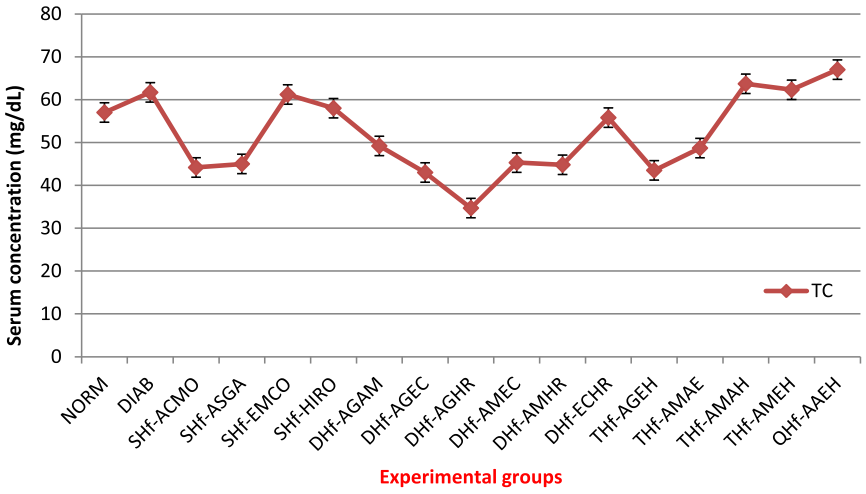

Fig. 2. Serum total cholesterol concentrations of normal, diabetic, and treated rats. ACMO = *Acanthus montanus*; ASGA = *Asystasia gangetica*; DHf = double herbal formula; EMCO = *Emilia coccinea*; HIRO = *Hibiscus rosasinensis*; NORM = normal; SHf = single herbal formula; THf = triple herbal formula. AGAM = combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *A. montanus*; AGEC = combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *E. coccinea*; AGHR = combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *H. rosasinensis*; AMEC = combined dose (ratio: 1:1 *w/w*) of *A. montanus* + *E. coccinea*; AMHR = combined dose (ratio: 1:1 *w/w*) of *A. montanus* + *H. rosasinensis*; ECHR = combined dose (ratio: 1:1 *w/w*) of E. coccinea + H. rosasinensis; AGEH = combined dose (ratio: 1:1:1 *w/w*) of *A. gangetica* + *E. coccinea* + *H. rosasinensis*; AMAE = combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *E. coccinea*; AMAH = combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *H. rosasinensis*; AMEH = combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *E. coccinea* + *H. rosasinensis*; AAEH = combined dose (ratio: 1:1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *E. coccinea* + *H. rosasinensis*; TC = total cholesterol.

TC of HrAGEH and HrAMAE were significantly ($p < 0.05$) lower than that of the NORM group, whereas serum TC of HrAMAH, HrAMEH, and HrAAEH were significantly different ($p < 0.05$) from the DIAB group.

Fig. 3 shows that $DIAB_{[VLDL\text{-}C]} = 14.5 \pm 2.13$ mg/dL was not significantly different ($p > 0.05$) from the $NORM_{[VLDL\text{-}C]} = 12.9 \pm 3.41$ mg/dL. Serum very low density lipoprotein-cholesterol (VLDL-C) of HyGR treated with SHf was within the range of 8.3 ± 2.14 mg/dL – 14.0 ± 2.42 mg/dL that was not significantly different ($p > 0.05$) from that of the NORM and DIAB groups. The $HrAGHR_{[VLDL\text{-}C]} = 10.5 \pm 0.84$ mg/dL and $HrAMEC_{[VLDL\text{-}C]} = 11.0 \pm 1.61$ mg/dL were significantly lower than that of DIAB and NORM groups. Conversely, the $HrAMHR_{[VLDL\text{-}C]} = 17.0 \pm 2.15$ mg/dL was significantly ($p < 0.05$) higher than the $DIAB_{[VLDL\text{-}C]} = 14.5 \pm 2.13$ mg/dL.

The VLDL-C of HyGR treated with THf and QHf was within the range of 10.8 ± 1.42 mg/dL – 14.5 ± 2.61 mg/dL, which was not significant different ($p > 0.05$) from that of the DIAB and NORM groups.

Fig. 4 shows that $DIAB_{[LDL\text{-}C]} = 27.9 \pm 1.73$ mg/dL was significantly higher than the $NORM_{[LDL\text{-}C]} = 11.5 \pm 1.22$ mg/dL, which represented an elevation of serum LDL-C by 2.4 folds. HyGR treated with SHf exhibited significant ($p < 0.05$) reduction in serum LDL-C concentration when compared with that of the DIAB group, except $HrACMO_{[LDL\text{-}C]} = 9.7 \pm 1.93$ mg/dL < $NORM_{[LDL\text{-}C]} = 11.5 \pm 1.22$ mg/dL; $p > 0.05$.

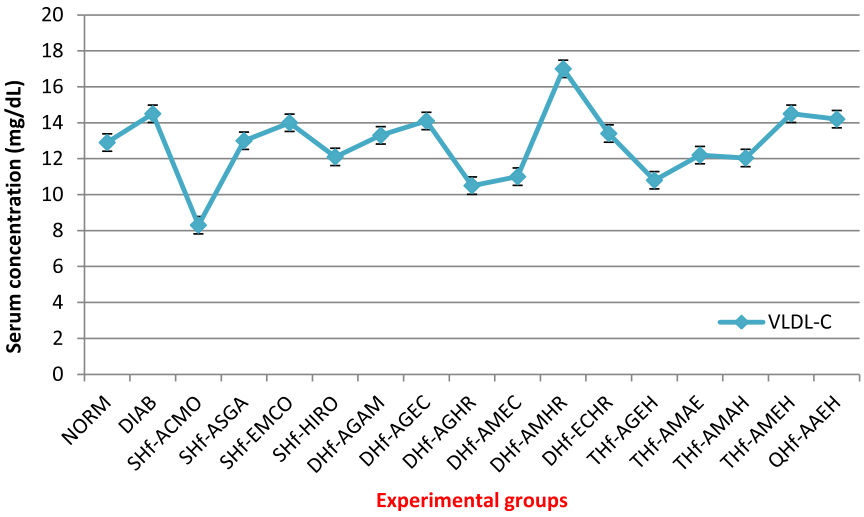

Fig. 3. Serum very low density lipoprotein-cholesterol concentrations of normal, diabetic, and treated rats. ACMO = *Acanthus montanus*; ASGA = *Asystasia gangetica*; DHf = double herbal formula; EMCO = *Emilia coccinea*; HIRO = *Hibiscus rosasinensis*; NORM = normal; SHf = single herbal formula; THf = triple herbal formula. AGAM = combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *A. montanus*; AGEC = combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *E. coccinea*; AGHR = combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *H. rosasinensis*; AMEC = combined dose (ratio: 1:1 *w/w*) of *A. montanus* + *E. coccinea*; AMHR = combined dose (ratio: 1:1 *w/w*) of *A. montanus* + *H. rosasinensis*; ECHR = combined dose (ratio: 1:1 *w/w*) of *E. coccinea* + *H. rosasinensis*; AGEH = combined dose (ratio: 1:1:1 *w/w*) of *A. gangetica* + *E. coccinea* + *H. rosasinensis*; AMAE = combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *E. coccinea*; AMAH = combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *H. rosasinensis*; AMEH = combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *E. coccinea* + *H. rosasinensis*; AAEH = combined dose (ratio: 1:1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *E. coccinea* + *H. rosasinensis*; VLDL-C = very low density lipoprotein-cholesterol.

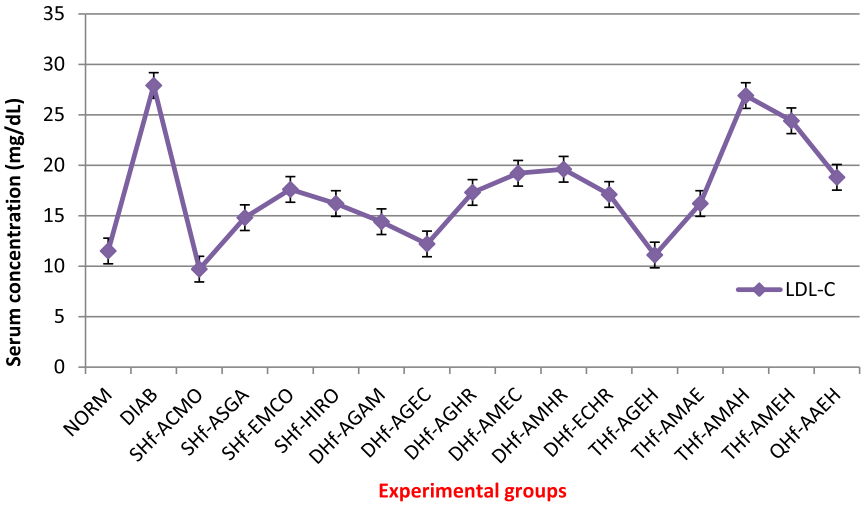

Fig. 4. Serum low density lipoprotein-cholesterol concentrations of normal, diabetic, and treated rats. ACMO = *Acanthus montanus*; ASGA = *Asystasia gangetica*; DHf = double herbal formula; EMCO = *Emilia coccinea*; HIRO = *Hibiscus rosasinensis*; NORM = normal; SHf = single herbal formula; THf = triple herbal formula. AGAM = combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *A. montanus*; AGEC = combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *E. coccinea*; AGHR = combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *H. rosasinensis*; AMEC = combined dose (ratio: 1:1 *w/w*) of *A. montanus* + *E. coccinea*; AMHR = combined dose (ratio: 1:1 *w/w*) of *A. montanus* + *H. rosasinensis*; ECHR = combined dose (ratio: 1:1 *w/w*) of *E. coccinea* + *H. rosasinensis*; AGEH = combined dose (ratio: 1:1:1 *w/w*) of *A. gangetica* + *E. coccinea* + *H. rosasinensis*; AMAE = combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *E. coccinea*; AMAH = combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *H. rosasinensis*; AMEH = combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *E. coccinea* + *H. rosasinensis*; AAEH = combined dose (ratio: 1:1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *E. coccinea* + *H. rosasinensis*; LDL-C = low density lipoprotein-cholesterol.

HyGR treated with DHf exhibited reduced serum LDL-C concentration when compared with that of the DIAB group. Furthermore, serum LDL-C of HyGR treated with DHf was within relatively narrow range of 12.2 ± 3.72 mg/dL – 19.6 ± 2.12 mg/dL. HyGR treated with THf: $HrAMAH_{[LDL\text{-}C]}$ = 26.9 ± 1.31 mg/dL and $HrAMEH_{[LDL\text{-}C]}$ = 24.4 ± 3.20 mg/dL were comparable with $DIAB_{[LDL\text{-}C]}$ = 27.9 ± 1.76 mg/dL; $p > 0.05$. Conversely, $HrAAEH_{[LDL\text{-}C]}$ = 18.8 ± 3.71 mg/dL was lower than that of $DIAB_{[LDL\text{-}C]}$ = 27.9 ± 1.73 mg/dL; $p < 0.05$.

The result presented in Fig. 5 show that $NORM_{[HDL\text{-}C]}$ = 29.5 ± 2.25 mg/dL > $DIAB_{[HDL]}$ = 12.1 ± 1.53 mg/dL; $p < 0.05$. Serum HDL-C of HyGR treated with SHf was within a relatively narrow range of 29.8 ± 1.23 mg/dL – 30.2 ± 2.12 mg/dL, which was not significantly different from the $NORM_{[HDL\text{-}C]}$ = 29.5 ± 2.25 mg/dL. Among the HyGR treated with DHf, $HrAGAM_{[HDL\text{-}C]}$ = 31.5 ± 2.75 mg/dL represented the peak value of serum HDL-C, whereas $HrECHR_{[HDL\text{-}C]}$ = 23.7 ± 2.01 mg/dL gave the lowest value. Similarly, serum HDL-C of HyGR treated with THf was within a relatively narrow range of 20.3 ± 2.31 mg/dL – 24.8 ± 3.22 mg/dL; $p > 0.05$. $HrAAEH_{[HDL\text{-}C]}$ = 44.3 ± 2.21 mg/dL represented 3.66-fold higher than the $DIAB_{[HDL\text{-}C]}$ = 12.1 ± 1.53 mg/dL; $p < 0.05$.

$DIAB_{[TAG]}$ = 72.5 ± 2.81 mg/dL > $NORM_{[TAG]}$ = 64.3 ± 3.15 mg/dL represented an 11.3% increase in serum TAG of the DIAB group; $p > 0.05$ (Fig. 6). Serum TAG of HyGR treated with SHf varied between 60.8 ± 3.5 mg/dL and 70.2 ± 5.0 mg/dL; $p > 0.05$. Although serum TAG of HyGR treated with DHf were lower than that of the DIAB group, the

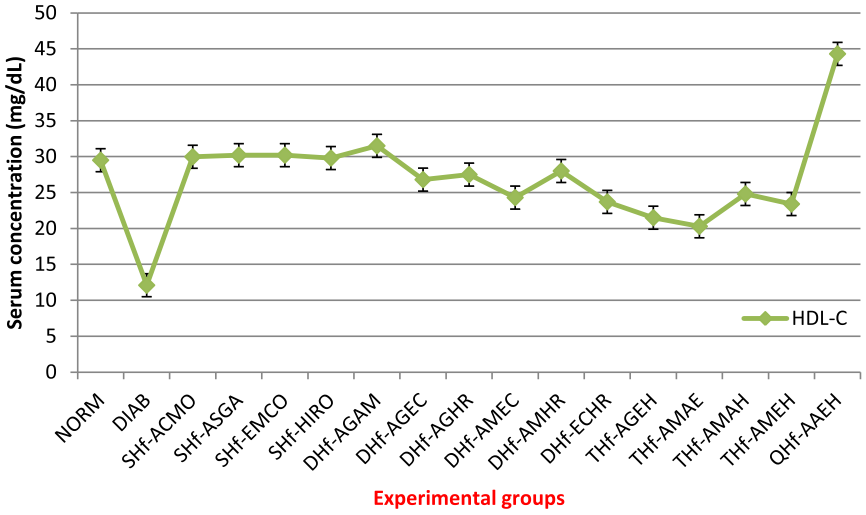

Fig. 5. Serum high-density lipoprotein-cholesterol concentrations of normal, diabetic, and treated rats. ACMO = *Acanthus montanus*; ASGA = *Asystasia gangetica*; DHf = double herbal formula; EMCO = *Emilia coccinea*; HIRO = *Hibiscus rosasinensis*; NORM = normal; SHf = single herbal formula; THf = triple herbal formula. AGAM = combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *A. montanus*; AGEC = combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *E. coccinea*; AGHR = combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *H. rosasinensis*; AMEC = combined dose (ratio: 1:1 *w/w*) of *A. montanus* + *E. coccinea*; AMHR = combined dose (ratio: 1:1 *w/w*) of *A. montanus* + *H. rosasinensis*; ECHR = combined dose (ratio: 1:1 *w/w*) of *E. coccinea* + *H. rosasinensis*; AGEH = combined dose (ratio: 1:1:1 *w/w*) of *A. gangetica* + *E. coccinea* + *H. rosasinensis*; AMAE = combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *E. coccinea*; AMAH = combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *H. rosasinensis*; AMEH = combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *E. coccinea* + *H. rosasinensis*; AAEH = combined dose (ratio: 1:1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *E. coccinea* + *H. rosasinensis*; HDL-C = high density lipoprotein-cholesterol.

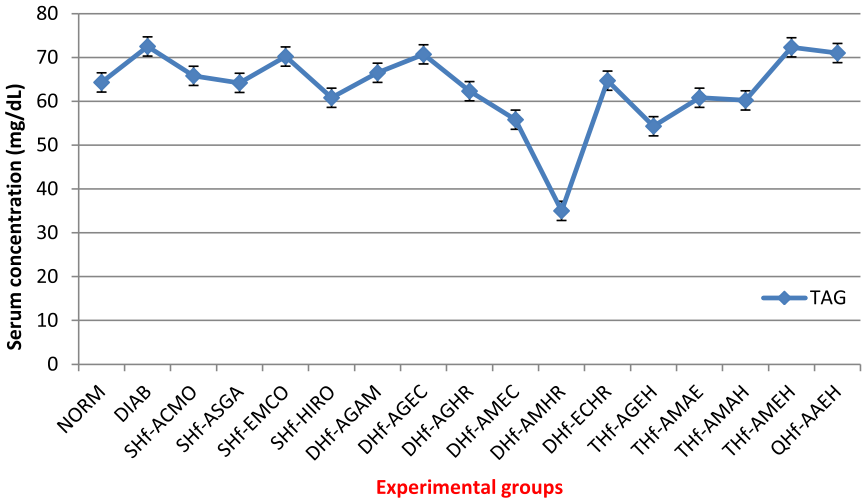

Fig. 6. Serum triacylglycerol concentrations of normal, diabetic, and treated rats. ACMO = *Acanthus montanus*; ASGA = *Asystasia gangetica*; DHf = double herbal formula; EMCO = *Emilia coccinea*; HIRO = *Hibiscus rosasinensis*; NORM = normal; SHf = single herbal formula; THf = triple herbal formula. AGAM = combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *A. montanus*; AGEC = combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *E. coccinea*; AGHR = combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *H. rosasinensis*; AMEC = combined dose (ratio: 1:1 *w/w*) of *A. montanus* + *E. coccinea*; AMHR = combined dose (ratio: 1:1 *w/w*) of *A. montanus* + *H. rosasinensis*; ECHR = combined dose (ratio: 1:1 *w/w*) of *E. coccinea* + *H. rosasinensis*; AGEH = combined dose (ratio: 1:1:1 *w/w*) of *A. gangetica* + *E. coccinea* + *H. rosasinensis*; AMAE = combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *E. coccinea*; AMAH = combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *H. rosasinensis*; AMEH = combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *E. coccinea* + *H. rosasinensis*; AAEH = combined dose (ratio: 1:1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *E. coccinea* + *H. rosasinensis*; TAG = triacylglycerol.

$HrAMEC_{[TAG]} = 55.8 \pm 3.22$ mg/dL and $HrAMHR_{[TAG]} = 35.0 \pm 2.43$ mg/dL were profoundly lower than that of the NORM group; $p < 0.05$. Likewise, $HrAGEH_{[TAG]} = 54.3 \pm 3.44$ mg/dL was significantly lower than that of the NORM group. In addition, $HrAMEH_{[TAG]} = 72.3 \pm 2.34$ mg/dL and $HrAAEH_{[TAG]} = 71.0 \pm 2.12$ mg/dL were not significantly different ($p > 0.05$) from the $DIAB_{[TAG]} = 72.5 \pm 2.81$ mg/dL.

Fig. 7 shows that HyGR treated with single and combinatorial herbal formulations exhibited reduced AI compared with the DIAB group; $p < 0.05$. In addition, AIs of HrAGAM, HrAGEC, HrAGHR, HrAMEC, HrAMHR, and HrAAEH were lower than that of the NORM group. For instance, $HrAGHR_{AI} = 0.26 \pm 0.22 < NORM_{AI} = 0.93 \pm 0.19$. Generally, HyGR treated with THfs exhibited the highest AIs compared with other HyGR treated with SHfs, DHfs, and QHf.

4. Discussion

Phytochemicals are bioactive principles that have been widely implicated in ameliorating vast array of clinical disorders and diseases[5,9] whose pathogenesis are remotely or directly connected with oxidative stress.[8,18,53] Bioactive principles of diverse plant origins have been experimentally described to exhibit glycemic control through varieties of mechanisms such as modulating the activity or gene expression of enzymes related to antioxidant,

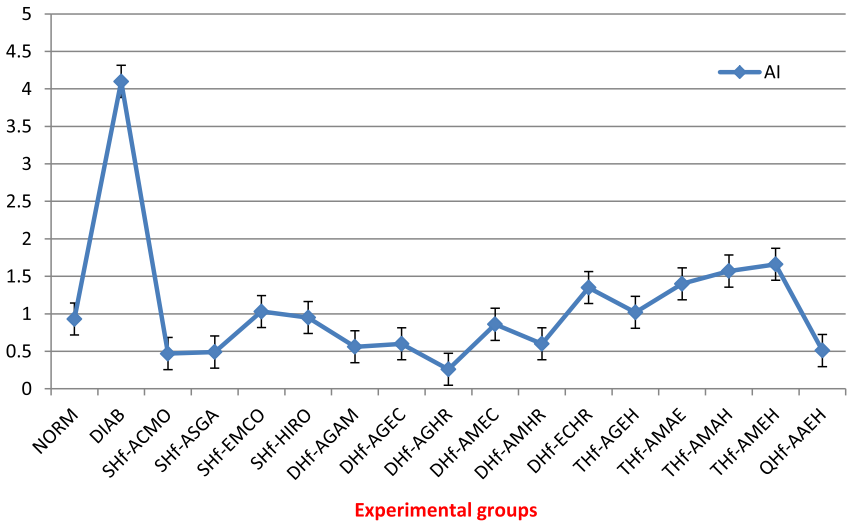

Fig. 7. Atherogenic indices of normal, diabetic, and treated rats. ACMO = *Acanthus montanus*; ASGA = *Asystasia gangetica*; DHf = double herbal formula; EMCO = *Emilia coccinea*; NORM = normal; SHf = single herbal formula; THf = triple herbal formula. AI = AGAM = combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *A. montanus*; AGEC = combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *E. coccinea*; AGHR = combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *H. rosasinensis*; AMEC = combined dose (ratio: 1:1 *w/w*) of *A. montanus* + *E. coccinea*; AMHR = combined dose (ratio: 1:1 *w/w*) of *A. montanus* + *H. rosasinensis*; ECHR = combined dose (ratio: 1:1 *w/w*) of *E. coccinea* + *H. rosasinensis*; AGEH = combined dose (ratio: 1:1:1 *w/w*) of *A. gangetica* + *E. coccinea* + *H. rosasinensis*; AMAE = combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *E. coccinea*; AMAH = combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *H. rosasinensis*; AMEH = combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *E. coccinea* + *H. rosasinensis*; AAEH = combined dose (ratio: 1:1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *E. coccinea* + *H. rosasinensis*; AI = atherogenic index.

glucose, and lipid homeostasis,[54] stimulating insulin secretion/mimicry,[8,24,55,56] improvement of hepatic glutathione concentration,[8,57] inhibition of intestinal α-glucosidase, pancreatic lipase, and cholesterol esterase activities,[58] facilitated muscle uptake of glucose,[59,60] regeneration/proliferation of β-cells,[24,61–64] and promoting insulin and adrenaline secretions and antioxidative capability.[8,65,66] Similarly, disarrangement in serum lipid profile engendered by lipemia in experimental animals have been successfully readjusted and brought to normalcy by the actions of bioactive principles of plant origins as previously demonstrated elsewhere.[49,58]

Earlier reviews[9,26] showed that alkaloids from varieties of plant species exhibited glycemic control. For instance, according to Jung et al[9] alkaloids extract from *Syzygium malaccense* L. (Myrtaceae) and *Penares schulzei* inhibited α-glucosidase activity. Furthermore, the alkaloids inhibited glycogen phosphorylase both *in vitro* and *in vivo* and stimulated basal glucose uptake rate in rat adipocytes. *Vinca rosea* L. (Apocynaceae), also known as Madagascar periwinkle, is widely cultivated in the tropics mainly for its alkaloids content, which possesses anticancer activities[67–69] and traditionally used for control diabetes from reports close to a century ago.[70] Fresh leaf juice of *Catharanthus roseus* has been reported to reduce blood glucose in normal and alloxan diabetic rabbits.[71] Similarly, Singh et al[72] demonstrated that leaves and twigs of *C. roseus* possess hypoglycemic activity in streptozotocin (STZ) induced diabetic rats. These reports corroborated the current suggestion that alkaloids in the herbal formulations served as an agent of glycemic control.

According to Najafian et al,[73] flavonoids are α-amylase inhibitors and the intermediary biosynthetic precursor, notably transchalcone, exhibit glycemic control in STZ-induced rat model of type I DM. They further noted that the hypoglycemic effect of flavonoids and, by extension, its derivatives ameliorated dyslipidemia in the diabetic rats. Zhou et al[74] had previously corroborated these reports in their investigations using flavonoids from lotus (*Nelumbo nuficera* Gaertn) leaf administered to diabetic mice. The antihyperlipidemic property of flavonoids has equally been confirmed in cancer engendered dyslipidemia.[75] In addition to these studies, Ma et al[76] proposed the possible mechanism by which flavonoids display these therapeutic properties. They noted that the capability of flavonoids from *Morus indica* to normalize blood lipids and glucose concentrations of high fat diet/low dose STZ-induced hyperlipidemic and hyperglycemic rats, were in connection with the upregulation of hepatic superoxide dismutase activity, reduction of hepatic malondialdehyde content, downregulation of hepatic CYP2E1 expression, and increase of glucose transporter 4 (GLUT-4) expression in skeletal muscle of the treated rats. Additionally, previous clinical studies proposed the application of citrus polymethoxylated flavones (PMFs) as agents of glycemic control and attenuation of insulin resistance,[77–79] which was subsequently demonstrated to be efficacious against metabolic syndrome and hyperlipidemia.[9,80,81] Similar studies by Jung et al[9] revealed that flavonoids caused increased adipocyte GLUT-4 activity, but decreased GLUT-2 expression, and increased hepatic/adipocyte peroxisome proliferator-activated receptor gamma (PPARγ) expression in treated animal models. Furthermore, flavonoids elicited decreased plasma and hepatic cholesterol levels through suppression of hepatic 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase and acyl CoA: cholesterol acyltransferase (ACAT) activities with increased fecal cholesterol.[9] Findings of the present study showed that leaf extracts used for the various experimental herbal formulations were relatively rich in flavonoids (Table 1), which obviously contributed to the observed glycemic control and antihyperlipidemic capabilities of the herbal formulations in the diabetic rats.

Similarly, saponins from *Solanum anguivi* fruit exhibited hypoglycemic, hypolipidemic, and antioxidant properties in alloxan-induced diabetic rats,[82] in which the therapeutic actions of saponins were described in the reports of Bhavsar et al.[83] Accordingly, they adduced that the bioactivity of saponins from *Helicteres isora* was related to increased gene expression of adipsin, GLUT-4, and PPARγ and reduced gene expression of glucose-6-phosphatase (G6Pase) and fatty acid binding protein 4 (FABP4) in treated diabetic mice. Also, steroidal saponin isolated from *Momordica charantia* L. was previously reported to possessing insulin-like activity.[84] The beneficial effect of saponins on hypercholesterolemia and

readjustments of lipoproteins metabolism-related disorders have been discussed elsewhere.[40]

The polyphenolic proanthocyanidin, commonly referred to as condensed tannin, has been reported to exhibit antioxidant activity[85,86] with antidiabetic property.[87] According to Yokozawa et al,[87] proanthocyanidin protected STZ-diabetic rats against hyperglycemia and related disorders as well as hyperlipidemia through modulation of general metabolism. In a related study, Velayutham et al[88] reported that tannin from *Ficus racemosa* attenuated oxidative stress and ameliorated hyperglycemia and dyslipidemia in diabetic rats. These previous findings, which corroborate the outcomes of the current study, were obvious indications that the therapeutic actions of alkaloids, flavonoids, saponins, and tannins were additive in the context of their collective capacities of these phytochemicals to exert glycemic control and antihyperlipidemic capabilities.[24,89]

Therefore, fluctuating capacities of the herbal combinations to alleviate hyperglycemia and hyperlipidemia in the animal models (Figs. 1–7) were outcomes of chemical interactions amongst the constituent phytochemicals of the various herbal formulations, which could either be synergistic or antagonistic as previously described.[90–95] The display of synergy or antagonism by the composite herbal extracts in ameliorating hyperglycemia and dyslipidemia depended on the type and number of individual herbal extract used in constituting the experimental herbal formulations. By implication, combination of the herbal extracts caused readjustments in the absolute concentrations of the bioactive principles, and by extension affected the nature and outcome of their interactions, which invariably dictated the therapeutic potentials of the various herbal formulations.

5. Conclusion

An overview of the current results (Figs. 1–6) showed that the capacities of the herbal formulations (SHfs, DHfs, THfs, and QHf) to exert glycemic control and reverse dyslipidemia did not follow predictable patterns in the animal models. However, the atherogenic indices of the treated HyGR were significantly ($p < 0.05$) lower than that of the untreated diabetic rats and comparable with the normal rats (Fig. 7).

Conflicts of interest

All authors declare no conflicts of interest.

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