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Comparative hypoglycemic activities of aqueous and ethanolic extracts of four medicinal plants (*Acanthus montanus, Asystasia gangetica, Emilia coccinea and Hibiscus rosasinensis*) in Type I diabetic rats

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ABSTRACT

Background: The present study ascertained the capacities of crude aqueous and ethanolic leaf extracts of *Acanthus montanus* (ACMO), *Asystasia gangetica* (ASGA), *Emilia coccinea* (EMCO), and *Hibiscus rosasinensis* (HIRO), as well as their combinatorial formulations to ameliorate hyperglycemia in Type I diabetic rats. **Materials and Methods:** Hyperglycemia was induced by single intraperitoneal injection of alloxan monohydrate in phosphate buffer saline (PBS) solution (pH = 7.4) dosage = 120 mg/kg; bw. Individual hyperglycemic rats (HyGR) received separate doses of either 20 mg/kg bw/24 h of ACMO, ASGA, EMCO or HIRO, as well as their combinatorial formulations (AAEH) for 14 days. Preparation of aqueous extracts (AQx) and ethanolic extracts (ETHx) of the four herbal samples was according to standard methods. Blood samples were drawn from 12 h post-fasted rats at regular intervals of 24 h for 14 days and measured for fasting blood glucose concentration (FBGC) using the glucose oxidase spectrophotometric method. **Results:** Cumulatively, ETHx of the herbal samples exhibited the greater capacity to lower FBGC in HyGR than that of the AQx. ETHx of AAEH exhibited the highest capacity to lower FBGC in HyGR by 53.55 ± 1.04%, whereas AQx of EMCO exhibited the lowest capacity to lower FBGC, which corresponded to 36.19 ± 0.88%. **Conclusion:** The study showed that ETHx of the herbal samples were comparatively more potent than the corresponding AQx as agents of glycemic control and for the management of hyperglycemia. Furthermore, the combination of the herbal extracts synergistically improved the therapeutic potentials of the individual herbal extracts.

KEY WORDS: Aqueous extracts, ethanolic extracts, hyperglycemia, medicinal plants

INTRODUCTION

Diabetes mellitus (DM) is the most common serious metabolic disorder considered to be one of the five leading causes of death in the world [1,2]. Among several pathophysiologic indicators [3-5], DM is primarily characterized by hyperglycemia. The multiple etiologies, classifications and complications of DM have been described elsewhere [6-9].

The use of plant materials as sources of food, cosmetics, and medicine for the benefit of human and domestic animals is as old as the existence of mankind. More so, a recent survey showed that 80% of Africa population relies on traditional herbal remedies, often referred to as alternative or complementary medicine in the industrialized countries, for the alleviation of pathologic conditions [10]. Medicinal plants contain active principles known to ancient and modern civilizations, for their healing properties, before the advent of synthetic therapeutic organic compounds at the dawn of the 19th century.

Due to plant diversity, the available active principles in plant materials exhibit diverse variability in terms of their physicochemical properties and corresponding medicinal usefulness [6,11]. The phytochemical contents and medicinal usefulness of *Acanthus montanus* (ACMO), *Asystasia gangetica* (ASGA), *Emilia coccinea* (EMCO), and *Hibiscus rosasinensis* (HIRO) have been mentioned by several authors [12-19].

The present study was restricted to chemically induced DM using animal models as previously described [1,7,20,21], which depicted the pathophysiology of Type I DM, and the capacities of crude aqueous and ethanolic leaf extracts of ACMO, ASGA, EMCO, and HIRO, as well as their combinatorial formulation to ameliorate hyperglycemia in experimental rats. In addition, the administration of combinatorial herbal formulations to hyperglycemic rats (HyGR) was on the premise that herbal concoctions from different plant species or genera might serve to potentiate the efficacy of the herbal extracts toward mitigating hyperglycemia.

MATERIALS AND METHODS

Collection and Preparation of Herbal Samples

Fresh leaves of ACMO (Nees) T. Anderson, EMCO G. Don and HIRO L. were collected from uncultivated lands in Umuamacha Ayaba Umaeze, Osisioma Ngwa Local Government Area (LGA), Abia State, Nigeria, whereas fresh leaves of ASGA L. T. Anderson were collected from Ubowuala, Emekuku, Owerri North LGA, Imo State, Nigeria. The four herbs were identified and authenticated by Dr. M. Ibe, School of Agriculture and Agricultural Technology, Federal University of Technology, Owerri. All the leaves were collected between the months of July and August, 2014.

The leaves of individual plants were washed with a continuous flow of distilled water for 15 min and allowed to dry at laboratory ambient temperature (24 ± 5°C). A 500 g part of each 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 a cold room (7 ± 3°C) for 24 h before pulverization. Next, the separate dried leaves were pulverized using Thomas-Willey milling machine (ASTM D-3182, INDIA), after which the ground samples were stored in air-tight plastic bottles with screw caps pending extraction.

Extraction of Herbal Samples

Preparation of ethanolic extracts (ETHx) of the four herbal samples was according to the methods previously described [19], whereas the corresponding aqueous extracts (AQx) was obtained according to the methods of Chikezie [22].

The separate extracts were reconstituted in phosphate buffered saline (PBS) solution (extract vehicle), osmotically equivalent to 100 g/L PBS (90.0 g NaCI, 17.0 g $Na_2HPO_4.2H_2O$, and 2.43 g $NaH_2PO_4.2H_2O$), before appropriated doses were administered to the experimental animals.

Experimental Animals

Male albino (Wistar) rats weighing between 150 g and 160 g were maintained at room temperature of 24 ± 5°C, 30-55% of relative humidity on a 12-h light/12-h dark cycle, with access to water and standard commercial feed (SCF) (Ewu Feed Mill, Edo State, Nigeria) *ad libitum* for 2 weeks 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).

Induction of Diabetes/Experimental Design

Hyperglycemia was induced in the experimental rats by intraperitoneal injection of 0.1 mol/L alloxan monohydrate (Sigma, St. Louis, MO., USA) as previously described by Ojiako and Chikezie [9] with minor modification to the dose administered to the rats (dose = 120 mg/kg; bw). A total of 72 male Wistar rats were allotted into 12 groups of 6 rats each. The animals were deprived of food and water for additional 16 h before the commencement of treatment as described elsewhere [8]. The animal groups were designated on the basis of treatments received at regular intervals of 24 h for 14 days.

- NORM: Normoglycemic rats received SCF + water *ad libitum* + 1.0 mL/kg of PBS.
- DIAB: HyGR (Diabetic Control) received SCF + water *ad libitum* + 1.0 mL/kg of PBS.
- Hr-AQx-ACMO: HyGR received SCF + water *ad libitum* + AQx of ACMO (20 mg/kg in PBS; i.p.).
- Hr-ETHx-ACMO: HyGR received SCF + water *ad libitum* + ETHx of ACMO (20 mg/kg in PBS; i.p.).
- Hr-AQx-ASGA: HyGR received SCF + water *ad libitum* + AQx of ASGA (20 mg/kg in PBS; i.p.).
- Hr-ETHx-ASGA: HyGR received SCF + water *ad libitum* + ETHx of ASGA (20 mg/kg in PBS; i.p.).
- Hr-AQx-EMCO: HyGR received SCF + water *ad libitum* + AQx of EMCO (20 mg/kg in PBS; i.p.).
- Hr-ETHx-EMCO: HyGR received SCF + water *ad libitum* + ETHx of EMCO (20 mg/kg in PBS; i.p.).
- Hr-AQx-HIRO: HyGR received SCF + water *ad libitum* + AQx of HIRO (20 mg/kg in PBS; i.p.).
- Hr-ETHx-HIRO: HyGR received SCF + water *ad libitum* + ETHx of HIRO (20 mg/kg in PBS; i.p.).
- Hr-AQx-AAEH: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1:1:1 *w/w*) of AQx of ACMO + ASGA + EMCO + HIRO (20 mg/kg in PBS; i.p.).
- Hr-ETHx-AAEH: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1:1:1 *w/w*) of ETHx of ACMO + ASGA + EMCO + HIRO (20 mg/kg in PBS; i.p.).

Blood volumes of 0.5 mL were drawn from 12 h post-fasted rats at regular intervals of 24 h for 14 days and measured for fasting blood glucose concentration (FBGC).

Fasting Plasma Glucose Concentration

FBGC was measured by the glucose oxidase spectrophotometric method according to Randox® kit manufacturer's procedure (Randox® Laboratories Ltd. Ardmore, United Kingdom).

Percentage Reduction in Fasting Blood Glucose Concentrations of Hyperglycemic Rats

Relative reduction in FBGC of the HyGR within the 14-day treatment period was calculated as the quotient of the

difference between FBGCs at the commencement of the experiment (i.e., t = day 0) and at the end of the experiment (i.e., t = day 14) and FBGC at t = day 0; thus.

$$\%RFBGC = \frac{(FBGC_{(day0)} - FBGC_{(day14)})}{FBGC_{(day0)}} \times 100 \quad (1)$$

%RFBGC: Percentage reduction in fasting blood glucose concentrations.

Statistical Analysis

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

RESULTS

An overview of Table 1 showed that ETHx of the four herbal samples gave relatively higher yield (g%; *w/w* ratio) than the corresponding AQx. The average cumulative yield of the herbal extracts was AQx = 14.39 g%, whereas ETHx = 17.07 g%. Figure 1 showed that FBGC of normoglycemic rats (NORM group), within the experimental time of 14 days, was relatively constant and ranged between 3.80 ± 0.24 mM/L and 4.83 ± 0.45 mM/L; $P > 0.05$. In addition, the FBGC of the untreated HyGR (DIAB group; Diabetic Control) was significantly ($P < 0.05$) higher than that of the NORM group. Specifically, FBGC of the DIAB group was within the range of 15.10 ± 1.11 mM/L – 19.91 ± 1.53 mM/L [Figure 1].

Table 1: Percentage yields of aqueous and ETHx of herbal samples

Herbal samples	Yield g%; *w/w* ratio	
	AQx	ETHx
ACMO	14.86	16.35
ASGA	12.02	16.69
EMCO	16.14	17.99
HIRO	14.55	17.23

AQx: Aqueous extract; ETHx: Ethanolic extract; ACMO: *Acanthus montanus* (Nees) T. Anderson; ASGA: Asystasia gangetica L.T. Anderson; EMCO: *Emilia coccinea* (SIMS) G. Don; HIRO: *Hibiscus rosasinensis* L.

Hr-AQx-ACMO was hyperglycemic ([FBGC] > 11.00 mM/L) for 12 consecutive days but became normoglycemic when $t \geq 13$ days. However, FBGC of Hr-AQx-ACMO was significantly ($P < 0.05$) higher than that of the NORM group. Likewise, Hr-ETHx-ACMO was normoglycemic at $t \geq 8$ days and FBGC of Hr-ETHx-ACMO was significantly ($P < 0.05$) higher than that of the NORM group within the experimental time of 14 days.

Figure 2 showed that Hr-AQx-ASGA was normoglycemic at $t \geq 6$ days. Estimations showed that FBGCs of both Hr-AQx-ASGA and Hr-ETHx-ASGA declined by ≈1.94 folds on the 14th day. Furthermore, at the end of the experimental time, FBGCs of Hr-AQx-ASGA and Hr-ETHx-ASGA were significantly ($P < 0.05$) higher than that of the NORM group. Furthermore, Hr-ETHx-ASGA was normoglycemic at $t \geq 7$ days.

Within the experimental time of 14 days, FBGC of Hr-AQx-EMCO ranged between 16.91 ± 0.91 mM/L and 10.54 ± 0.79 mM/L, whereas FBGC of Hr-AQx-EMCO gave: 15.91 ± 0.83 mM/L - 9.00 ± 0.71 mM/L. Hr-AQx-EMCO was normoglycemic at $t \geq 10$ days, whereas Hr-ETHx-EMCO was normoglycemic at $t \geq 11$ days. At the end of the experimental time, FBGC of Hr-AQx-EMCO represented 36.20% reduction in circulating blood glucose concentration. By the same estimation, FBGC of Hr-ETHx-EMCO corresponded to 43.43% reduction in circulating glucose concentration. FBGCs of Hr-AQx-EMCO and Hr-ETHx-EMCO were significantly ($P < 0.05$) higher than that of the NORM group [Figure 3].

Figure 4 showed that Hr-AQx-HIRO and Hr-ETHx-HIRO were normoglycemic $t \geq 9$ days and $t \geq 6$ days, respectively.

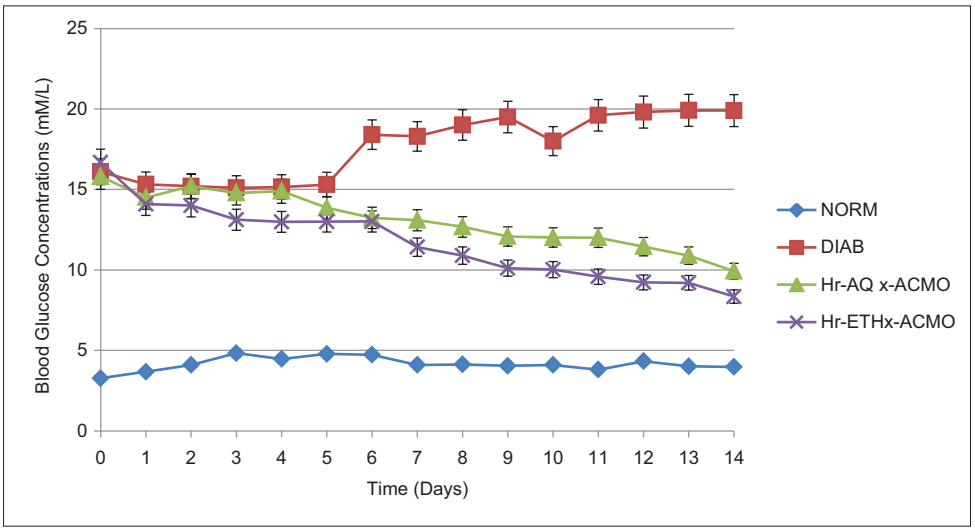

Figure 1: Comparative fasting blood glucose concentrations of normoglycemic, untreated hyperglycemic and hyperglycemic rats treated with aqueous and ethanolic extracts of *Acanthus montanus* (ACMO)

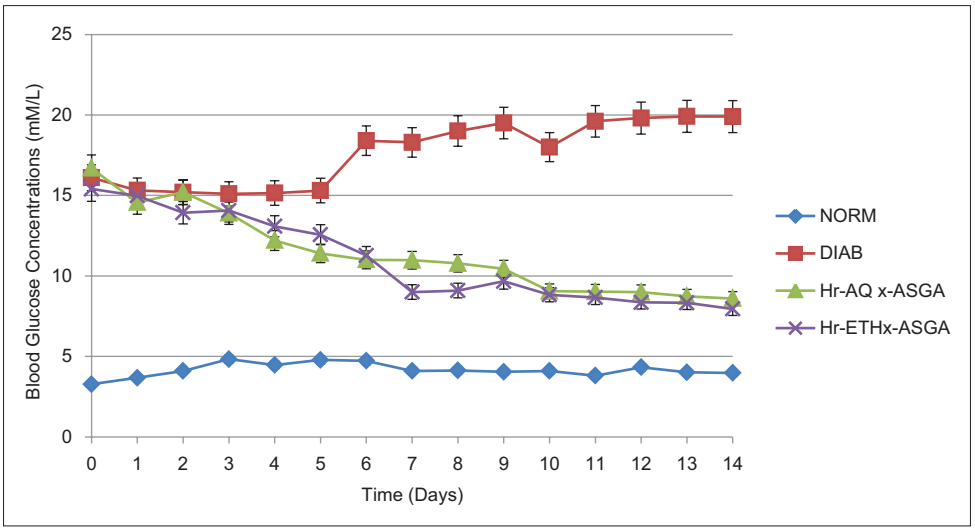

Figure 2: Comparative fasting blood glucose concentrations of normoglycemic, untreated hyperglycemic and hyperglycemic rats treated with aqueous and ethanolic extracts of *Asystasia gangetica* (ASGA)

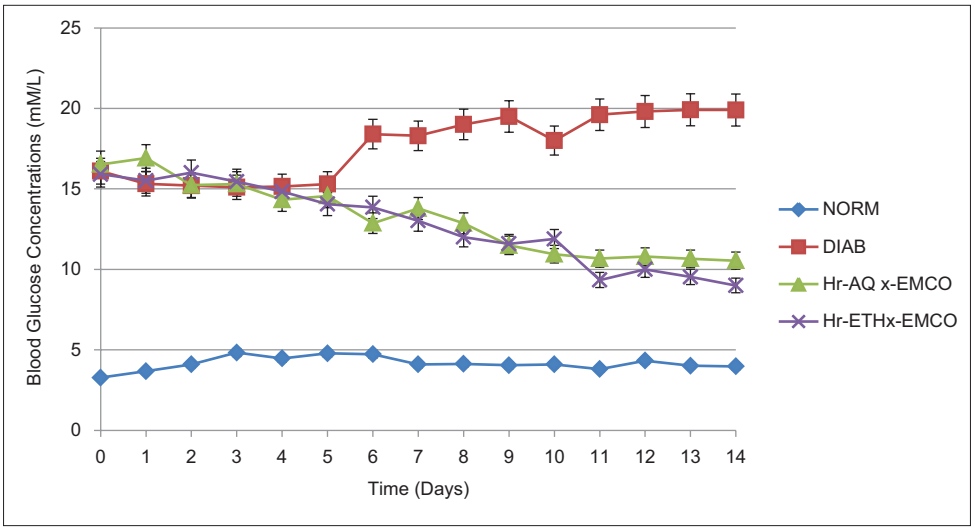

Figure 3: Comparative fasting blood glucose concentrations of normoglycemic, untreated hyperglycemic and hyperglycemic rats treated with aqueous and ethanolic extracts of *Emilia coccinea* (EMCO)

FBGC of Hr-AQx-HIRO declined by 1.53 folds on the 14th day, whereas that of Hr-ETHx-HIRO declined by 1.93 folds within the same experimental period. FBGCs of Hr-AQx-HIRO and Hr-ETHx-HIRO were significantly ($P < 0.05$) higher than that of the NORM group at $t = 14$ days.

Figure 5 showed that Hr-AQx-AAEH and Hr-ETHx-AAEH were normoglycemic at t ≥ 4 days. Reduction in FBGCs at t = 14 days were as follows: Hr-AQx-AAEH = 1.87 folds and Hr-ETHx-AAEH = 2.15 folds. In addition, a cursory view of Figure 5 showed that the lowest FBGC occurred at $t \geq 11$ days; thus, Hr-AQx-AAEH$_{[FBGC]}$ = 8.10 ± 0.08 mM/L and Hr-ETHx-AAEH$_{[FBGC]}$ = 6.88 ± 0.81 mM/L; $P > 0.05$. FBGCs of Hr-AQx-AAEH and Hr-ETHx-AAEH were significantly ($P < 0.05$) higher than that of the NORM group.

Finally, an overview of Figures 1-5 showed that AQx and ETHx of the herbal samples caused a reduction in FBGCs in HyGR in the order: ETHx > AQx.

The relative reduction in FBGC in HyGR following the administration of the herbal extracts within the 14-day treatment period is presented in Table 2. Specifically, Table 2 showed that the capacities of the herbal extracts to reduce FBGC in HyGR were in the following order: Hr-ETHx-AAEH > Hr-ETHx-ACMO > Hr-AQx-ASGA > Hr-ETHx-ASGA > Hr-ETHx-HIRO > Hr-AQx-AAEH > Hr-ETHx-EMCO > Hr-AQx-ACMO > Hr-AQx-HIRO > Hr-AQx-EMCO.

DISCUSSION

The present study showed that herbal extracts and its combinatorial formulations exhibited blood glucose lowering effect in HyGR, which conformed to previous reports [6,7,9,21,23, 24]. According to those reports, the capacity of plant extracts to ameliorate hyperglycemia in experimental

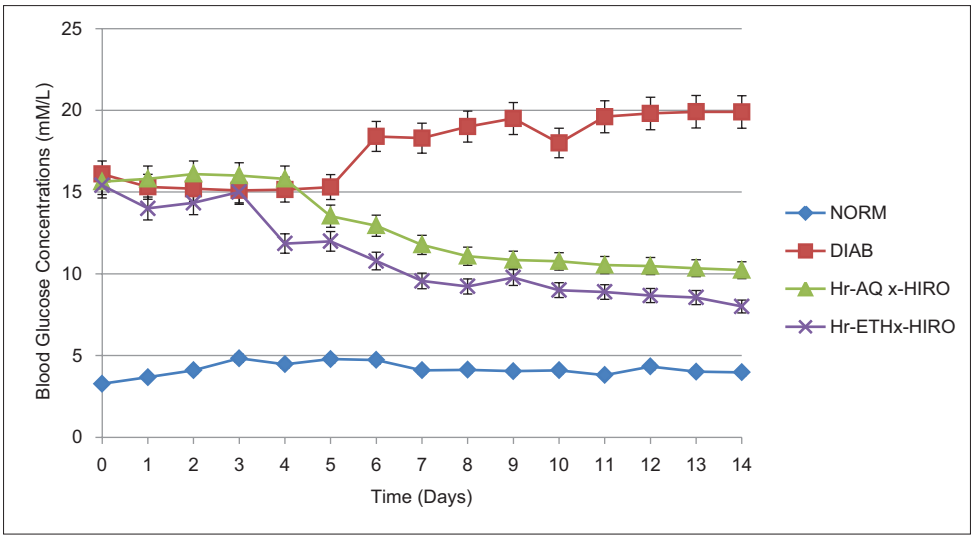

Figure 4: Comparative fasting blood glucose concentrations of normoglycemic, untreated hyperglycemic and hyperglycemic rats treated with aqueous and ethanolic extracts of Hibiscus rosasinensis (HIRO)

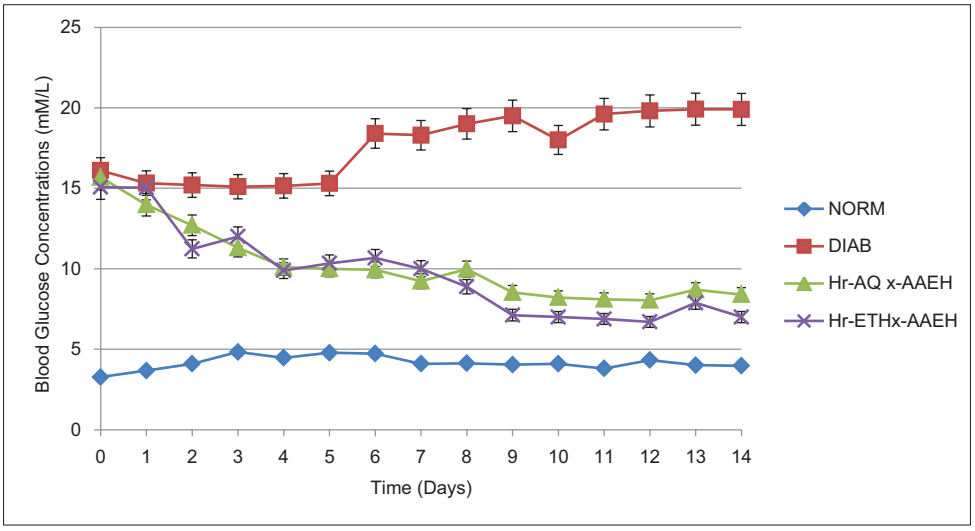

Figure 5: Comparative fasting blood glucose concentrations of normoglycemic, untreated hyperglycemic and hyperglycemic rats treated with aqueous and ethanolic extracts of combinatorial formulations of four herbal samples (AAEH)

animals was attributed to their phytochemical contents; notably the flavonoids, alkaloids, polyphenols, terpenoids, coumarins, and several other bioactive constituents, which in turn dictated the cumulative pharmacognostic potencies of the herbal formulations. Anti-diabetic bioactive principles exhibit a variety of biologic activities and therapeutic mode of actions that have been previously described [6,9,25]. Accordingly, the relatively high flavonoid content, in particular, in addition to the presence and mutual effects of variety of anti-diabetic phytochemicals in the herbal extracts [19] obviously contributed to their divergent capacities to exert hypoglycemic effect in the experimental rats as represented in the present study [Table 2].

Likewise, studies have shown that the absolute concentrations and available bioactive principles from herbal samples depended on the nature of solvent used in the extraction process, which among other experimental factors dictated the hypoglycemic potency of the medicinal preparations [1,25,26]. The results of the present study showed that EHx of the herbal samples was comparatively more efficacious than their corresponding AQx as an agent of glycemic control and for the management of hyperglycemia.

The comparative capacity of the combinatorial herbal extracts to exert high glycemic control in HyGR, as exemplified by Hr-ETHx-AAEH, appears to suggest synergy among the anti-diabetic bioactive principles of the component herbal extracts. Previous reports have confirmed that combinations of different herbal extracts in solution altered the biologic and pharmacologic properties of the constituent bioactive principles as a result of inter-phytochemical interactions [27,28].

Table 2: Percentage reduction in fasting blood glucose concentrations of HyGR administered with herbal extracts within experimental time (0 day ≥ *t* ≥14 days)

Rat groups	Reduction in FBGC (%)
Hr-AQx-ACM0	$37.21\pm0.75^{f,g,h}$
Hr-ETHx-ACM0	$49.97\pm1.05^{a,b}$
Hr-AQx-ASGA	$48.47\pm1.01^{a,b,c}$
Hr-ETHx-ASGA	$48.37\pm0.93^{a,b,c,d}$
Hr-AQx-EMC0	$36.19\pm0.88^{f,g,h,i,j}$
Hr-ETHx-EMC0	$43.43\pm0.95^{b,c,d,e,f,g}$
Hr-AQx-HIR0	$36.61\pm0.88^{f,g,h,i}$
Hr-ETHx-HIR0	$48.09\pm1.05^{a,b,c,d,e}$
Hr-AQx-AAEH	$46.43\pm0.98^{a,b,c,d,e,f}$
Hr-ETHx-AAEH	53.55 ± 1.04^{a}

The mean (X)±SD of six (n=6) determinations. Means in the column with the same letter are not significantly different at $P>0.05$,
SD: Standard deviation, HyGR: Hyperglycemic rats,

CONCLUSION

In that regard, results of the present study showed that the combination of the various herbal extracts synergistically improved the therapeutic potentials of the individual herbal extracts as agents of glycemic control. Furthermore, the study showed that ETHx of the herbal samples was comparatively more potent than the corresponding AQx as agents of glycemic control and for the management of hyperglycemia. Nevertheless, the aqueous and ethanolic leaf extracts of the four medicinal plants and their combinatorial formulations, in the present crude form, did not restore full therapeutic benefits to the HyGR within the experimental time.

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