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Research Article

Comparative Hypoglycemic Property of Aqueous and Ethanolic Extracts of *Viscum album* (Mistletoe) and Their Effects on Body and Organ Weights of Diabetic Rats (*Rattus norvegicus*).

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ABSTRACT: Plants are veritable sources of bioactive principles with profound therapeutic and medicinal value. The present study sought to determine, on comparative basis, the capacities of aqueous and ethanolic leaf extracts of *Viscum album* to ameliorate hyperglycemia and their effects on the body and visceral organ weights of experimental animals. Preparation of aqueous and ethanol/water (ratio 2:1; *v/v*) extracts of *V. album* was according to standard methods. Hyperglycemia was induced in 12 h fasted animals by intra-peritoneal injection of 0.1 mol/L alloxan monohydrate in phosphate buffer saline (PBS) solution (pH =7.4) at a dose of 140 mg/kg per body weight (*b/w*). Blood samples were drawn from the rats at regular intervals of 2 days for 14 days and measured for fasting blood glucose (FBG) using spectrophotometric methods. The body weight (*b/w*), pancreas, liver, kidney, heart and spleen of the rats were measured with electronic weighing balance. Comparative analyses showed that the aqueous leaf extract of *V. album* exhibited greater capacity to lower % FBG levels than the corresponding ethanolic leaf extract within the experimental time intervals of 0 day $\leq t \leq$ 6 days; $p > 0.05$. In contrast, at $t >$ 6 days, ethanolic leaf extract of *V. album* showed greater capacity to lower % FBG levels than corresponding the aqueous leaf extract; $p < 0.05$. Hyperglycemic rats without glycemic control exhibited 6.55 ± 1.08 % loss in *b/w*. Increases in kidney and heart weights of the test groups were not significantly different ($p > 0.05$) from the control (group C-N). Untreated diabetic rats showed significant ($p < 0.05$) increases in pancreas, liver and spleen weights compared to the group C-N. The therapeutic thresholds of the leaf extracts might be in connection with pharmacognostic dynamics coupled with toxicological outcomes of the administered leaf extracts.

KEYWORDS: Fasting blood glucose, *Rattus norvegicus*, visceral organ, *Viscum album,* diabetes mellitus, mistletoe.

INTRODUCTION

From time immemorial, plants have been veritable sources of numerous bioactive principles with profound therapeutic and medicinal value. The utilization of animal models to evaluate ameliorative potentials of herbal decoctions to diverse pathologic states and diabetes mellitus (DM) in particular have been severally reported.[1–11] Most often, induction of DM is achieved by intra-peritoneal injection of alloxan or streptozotocin salt solution to experimental animals, which engenders a classic case of Type I DM.[6–8,12] Thus, these experimental models are restrictive, in the sense that the scope of investigation is targeted at Type I DM in exclusion to other types of DM described elsewhere.[2,4,13] However, encouraging results obtained from studies of chemically induced diabetic animals have provided insight into the prospects of herbal remedies for the treatment and management of DM. Furthermore, the option of substituting conventional anti-diabetic preparations such as insulin, sulfonylureas, biguanides, thiazolidinedione and their derivatives for herbal products will eliminate avoidable adverse reactions/toxic side effects[14] and high cost associated with the use of these orthodox therapeutic drugs.[15,16]

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Viscum album (Mistletoe) is a partial parasitic plant to several varieties of temperate and tropical trees in Africa, Asia, Europe and Australia.[3,17,18] Phytochemical analyses by previous researchers have shown that *V. album* contains a wide variety of pharmacological active principles of medical and toxicological importance.[3,4,18–23] However, the potency of *V. album* decoction depends on the host plant,[24] geographical regions and environmental conditions of source.[17] Although there are several previous reports on the hypoglycemic potency of *V. album*, the present study sought to carry out, on comparative basis, the capacities of aqueous and ethanolic leaf extracts of *V. album* to ameliorate hyperglycemia in concurrence with the effects of the two types of extract on body and visceral organ weights of experimental animals.

From another perspective, multidimensional alterations in cellular metabolism and ensuing pathophysiology of DM are manifested in the form of tissue/organ dysfunctional outcomes and weight adjustments. Also, changes in body and organ weights of the experimental animals could serve as a basis for assessment of the possible underlying toxicologic effects resulting from the administration of *V. album* extracts.

MATERIALS AND METHODS

***Collection and preparation of plant extracts*:** Whole plants of *Viscum album* growing on palm oil trees were harvested from a botanical garden located within the environment of Federal University of Science and Technology, Owerri, Nigeria, between the months of July and August, 2012. The plant specimen was identified and authenticated by Dr. F.N. Mbagwu, Department of Plant Science and Technology, Imo State University, Owerri. A voucher specimen was deposited in the Herbarium for reference purpose. Preparation of aqueous and ethanol/water (ratio 2:1; *v/v*) extracts of *V. album* was according to the methods previously described by Ibegbulem and Chikezie,[25] with some modifications. The yield of the two separate *V. album* extracts was calculated to be: aqueous extract = 3.8% *w/w* and ethanol/water (ratio 2:1; *v/v*) extract = 5.3% *w/w*. The extracts were finally suspended in phosphate buffered saline (PBS) solution osmotically equivalent to 0.9 g/100 mL NaCI {NaCI (9.0 g), $Na_2HPO_4.2H_2O$ (1.71 g) and $NaH_2PO_4.2H_2O$ (0.243 g)/100 mL; pH = 7.4}, stored at 4°C for 24 h and subsequently administered to the rats.[25]

***Experimental animals*:** Healthy male rats (*Rattus norvegicus*) of 5–6 weeks old and weighing between 105-135 g were obtained from the animal house of Federal University of Science and Technology, Owerri, Nigeria. The rats were fed with standard commercial feed (SCF) (Ewu Feed Mill, Edo State, Nigeria) and water, in a well-ventilated stainless steel cages. After randomization on weight bases, the rats were allowed to acclimatize for 7 days at an ambient room temperature of 25±5°C, 30–55% of relative humidity on a 12-h light/12-h dark cycle. Animal handling was in accordance with the standard principles of laboratory animal care of the United State National Institute of Health (NIH, 1978).

***Induction of hyperglycemia and study design*:** Hyperglycemia was induced in 12 h fasted animals by intra-peritoneal injection of 0.1 mol/L alloxan monohydrate (Sigma, St. Louis, USA) in phosphate buffer saline (PBS) solution (pH =7.4) at a dose of 140 mg/kg per body weight (*b/w*). Hyperglycemia was confirmed in rats 72 h after alloxan treatment: Rats with fasting blood glucose (FBG) concentration > 11.0 mmol/L were considered to be hyperglycemic and selected for the study.

A total of thirty (30) rats were divided into five (5) weight cohorts of six ($n = 6$) each as follows:

- Group C-N: Control-Normal rats received SCF + PBS (Vehicle; 1.0 mL/kg; *b/w*/24 h, i. p.) for 14 days.
- Group C-H: Control-Hyperglycemic rats received SCF + PBS (Vehicle; 1.0 mL/kg; *b/w*/24 h, i. p.) for 14 days.
- Group H-AQ-E: Hyperglycemic rats received SCF + aqueous extract of *V. album* (400 mg/kg; *b/w*/24 h, i. p.) for 14 days.
- Group H-ETH-E: Hyperglycemic rats received SCF + ethanol/water (ratio 2:1; *v/v*) extract of *V. album* (400 mg/kg; *b/w*/24 h, i. p.) for 14 days.
- Group H-STD-G: Hyperglycemic rats received SCF + glibenclamide (5 mg/kg/24 h, i. p.) for 14 days.

***Measurement of fasting blood glucose*:** Blood samples were drawn from the tail of the rats at regular intervals of 2 days for 14 days and measured for fasting blood glucose (FBG). Determination of FBG was by glucose oxidase method according to the Randox® kit manufacturer's procedure (Randox® Laboratories Ltd. Ardmore, United Kingdom).

***Measurement of body/visceral organ weights*:** The *b/w* of the rats in the various experimental groups was measured with an electronic weighing balance {Digital Precision Weighing Balance (JCS-QC03) – China} at regular intervals of 2 days for 14 days. The visceral organs, namely, pancreas, liver, kidney, heart and spleen of the corresponding animal groups were removed after

sacrificing the animals on the 14th day and rinsed in 10% formolsaline {(formalin composed of 40 mL formaldehyde + 100 mL distilled water); 10 mL aliquot of formalin + 90 mL normal saline} solution to remove blood constituents. The organs were placed on blotting papers and allowed to dry at room temperature (25±5°C) for 2 h before weighing accordingly. Organ weight was reported in grams per *b/w* (g/*b/w*).

***Statistical analyses*:** The data collected were expressed in means (X) ± SD and analyzed in one-way ANOVA and Least Significance Difference (LSD). The comparison was made between groups and significance was established by ANOVA at 95% confidence level. Difference of $p < 0.05$ was considered statistically significant.

RESULTS

The average FBG concentration of group C-N was between 5.92±0.04 and 6.20±0.02 mmol/L. Within the experimental time of 14 days, the four test groups (C-H, H-AQ-E, H-ETH-E and H-STD-G) exhibited elevated relative FBG concentration (%FBG) compared to the group C-N. However, an overview of Figure 1 showed time-dependent declining levels of elevated %FBG in the experimental groups treated with leaf extracts of *V. album* and glibenclamide accordingly.

Contrary, %FBG of untreated hyperglycemic rats (group C-H) was perpetually elevated within the range of 148.01±0.92–169.042%. Specifically, the capacities of *V. album* leaf extracts and glibenclamide to cause reduction in %FBG levels was in the order: H-STD-G > H-ETH-E > H-AQ-E, which corresponded to H-STD-G = 100.06%, H-ETH-E = 60.89% and H-AQ-E = 42.02% ($p < 0.05$) reduction in %FBG levels within the experimental time of 24 days. Comparative analyses showed that aqueous leaf extract of *V. album* exhibited greater capacity to lower %FBG levels than the corresponding ethanolic leaf extract within the experimental time intervals of 0 day ≤ t ≤ 6 days; $p > 0.05$. Conversely, time intervals of 6 days ≤ t ≤ 14 days showed that reducing %FBG levels was H-ETH-E > H-AQ-E; $p < 0.05$. However, at $t <$ 4 days, H-AQ-E and H-ETH-E exhibited poor glycemic control. It is worthwhile to note that group H-STD-G showed normoglycemic status at $t \geq 10^{th}$ day of treatment with [FBG] in the range of 6.83±0.02–9.45±0.04 mmol/L (group $C\text{-}N_{[FBG]}$ ranged between 5.92±0.04–6.20±0.02 mmol/L). At $t = 14^{th}$ day, FBG concentration of group H-AQ-E did not indicate normoglycemic status; $H\text{-}AQ\text{-}E_{[FBG]}$ = 12.06±0.02 mmol/L ($p < 0.05$), in contrast to $H\text{-}ETH\text{-}E_{[FBG]}$ = 9.14±0.04 mmol/L ($p < 0.05$) and $H\text{-}STD\text{-}G_{[FBG]}$ = 5.81±0.04 mmol/L ($p > 0.05$). Furthermore, the rate of reduction in %FBG level in the presence of glibenclamide was more rapid within the first four days of the drug administration, whereas the rate of

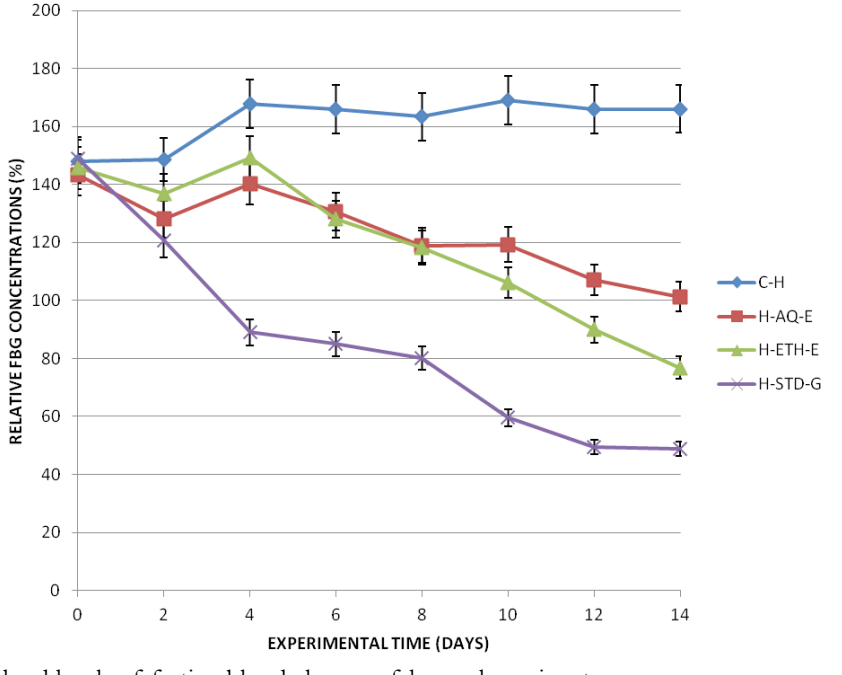

Figure 1. Comparative blood levels of fasting blood glucose of hyperglycemic rats.

glycemic control in groups H-AQ-E and H-ETH-E was more profound after 4 days of treatment with the two leaf extracts of *V. album*.

Figure 2 showed that group C-N exhibited increase in *b/w* by 6.88±1.24% at the end of the experimental time, whereas hyperglycemic rats without glycemic control exhibited 6.55±1.08% loss in *b/w*. The loss in *b/w* of group C-H was more rapid within the period of 0–4 days than subsequent stage of the experimental time. Figure 2 shows that the initial loss in *b/w* of corresponding hyperglycemic rats treated with the two leaf extracts of *V. album* and glibenclamide subsequent to sustained increase in *b/w*. For instance, increasing *b/w* of the experimental animals was observed in groups H-AQ-E, H-ETH-E and H-STD-G after the 2nd, 4th and 2nd days of treatment respectively. Finally, at t = 14 days, the relative change in *b/w* of the five experimental groups was in the order: C-N = 6.88±1.24% > H-STD-G = 4.22±1.01% > H-ETH-E = 2.72±0.99% > H-AQ-E = 0.92±1.22% > C-H = -7.56±0.95% (Figure 2).

Generally, there were various increased levels of organ weights in the four test groups except a slight decreased liver weight of group H-STD-G ($p > 0.05$) compared to group C-N (Table 1). In addition, increases in kidney and heart weights of the four test groups were not significantly different ($p > 0.05$) from group C-N. Group C-H showed significant ($p < 0.05$) increases in pancreas, liver and spleen weights compared to the group C-N. Conversely, increases in pancreas weight in the groups H-AQ-E, H-ETH-E and H-STD-G were not significantly different ($p < 0.05$) in relation to the pancreas weight of group C-N. However, the liver weights of the groups H-AQ-E and H-ETH-E were significantly ($p < 0.05$) higher than the normal/control group. Finally, increases in the weights of the spleens of the various test groups were significantly ($p < 0.05$) higher than the group C-N.

DISCUSSION

The capability of aqueous and ethanolic extracts of *V. album* to exert glycemic control in hyperglycemic rats, in similar patterns with the standard diabetic drug-glibenclamide, conformed to several previous reports into other plant extracts in this regard elsewhere.[8,9,17,26] The comparative poor capacity of the two extracts to exert glycemic

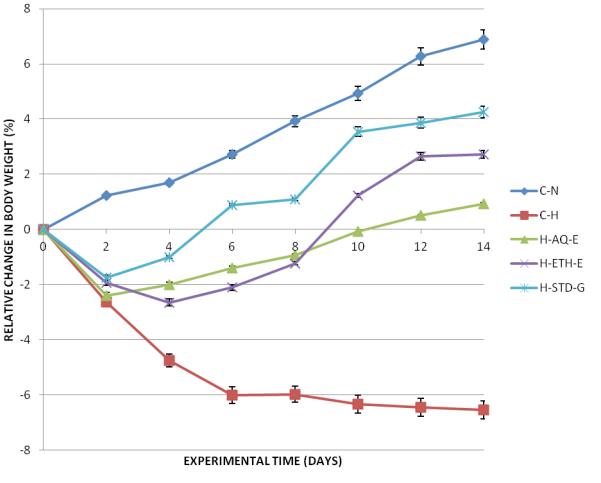

Figure 2. Percentage changes in body weights of experimental rats.

Table 1: Relative visceral organ weights of experimental rats.

Group	Organ Weight g/*b/w* (×10^{-2})					
	Pancreas	Liver	L. Kidney	R. Kidney	Heartt	Spleen
C-N	1.00±0.03^{b,c,d,e}	3.80±0.13^{c,d}	0.43±0.01	0.40±0.01	0.32±0.02	0.30±0.04^{e}
C-H	1.56±0.04^{a}	4.23±0.20^{a,b}	0.58±0.02	0.55±0.01	0.30±0.03	0.86±0.02^{a}
H-AQ-E	1.02±0.03^{b,c,d}	4.09±0.04^{a,b,c}	0.46±0.02	0.47±0.02	0.38±0.01	0.67±0.02^{a,b,c}
H-ETH-E	1.27±0.06^{a,b}	4.43±0.04^{a}	0.49±0.01	0.48±0.01	0.42±0.02	0.62±0.04^{a,b,c,d}
H-STD-G	1.11±0.03^{b,c}	3.71±0.02^{d,e}	0.45±0.01	0.46±0.01	0.32±0.02	0.70±.04^{a,b}

Means in the column with the same alphabet or without superscript are not significantly different ($p < 0.05$) according to LSD.

control at the early stage of treatment (at $t < 4$ days), which was at variance following the administration of glibenclamide, seems to suggest that the mode of action of bioactive principle of the leaf extracts was different from that of glibenclamide. Glibenclamide, like other second generation sulphonylurea anti-diabetic drugs, act by stimulating pancreatic β-cells to secrete insulin that in turn promotes cellular uptake of glucose.[27,28] Previous studies have shown that blood glucose lowering effects of plant extracts may be facilitated by insulin mimicry,[10,29,30] stimulation of insulin biosynthesis/secretion,[31] neutralization of free radicals,[11,21,26,32] progressive regeneration of the β-cells sequel to damage by diabetogenic agents.[5,33,34] Also, there are extra-pancreatic mechanisms involving the suppression of gluconeogenic enzymes, promotion of glycogen biosynthesis,[35–38] and reduction of intestinal glucose absorption[39] but with concomitant enhanced glucose uptake by peripheral tissue.[7]

Studies have also shown that the nature of solvent used for extraction of bioactive principles from plant materials, amongst other factors, is a determining factor for extract potency.[8,9,40] For instance, comparative studies by Pierre *et al.,*[11] noted that methanolic extract of *Bersama engleriana* produced more hyperglycemia alleviating effect than the aqueous extract. In similar manner, ethanolic leaf extract of *V. album* showed a greater capacity to ameliorate hyperglycemia compared to the equivalent dose of the aqueous leaf extract. Thus, the observations above was an indication that although hypoglycemic elements are present in both aqueous and ethanolic leaf extracts of *V. album*, the ethanolic extract appears to contain relatively more combinations of hypoglycemic bioactive principles with corresponding greater potency than the aqueous extract.

Undesirable *b/w* loss is associated with the Type I DM[41,42] on account of distortions in metabolic events following clinical presentation of this pathologic state. The improvements in *b/w* of the various experimental groups (H-STD-G, H-ETH-E and H-AQ-E) in contrasted to the untreated group (C-H) were an obvious indication of the therapeutic benefits derivable from leaf extracts of *V. album*. In conformity with the present study, several researchers have used the improvement in *b/w* as a basis for confirmation of ameliorative property of plant materials in diabetic animal models.[5,6,11,26,41] According to Pierre *et al.,*[11] the capacities of aqueous and methanolic extracts of *B. engleriana* to readjust FBG to normal values in nicotinamide/streptozotocin-induced diabetic rats correlated fairly well with corresponding improvements of their *b/w*. In addition, the pattern of improvement in *b/w* of groups H-ETH-E and H-AQ-E compared fairly well the rats treated with the standard anti-diabetic drug (group H-STD-G) (Figure 2).

Visceral organ weight was reported here (Table 1) on the basis of organ-to-body weight ratio as against absolute organ weight in order to eliminate the variability that may arise due to fluctuations and inconsistencies in *b/w* of experimental animals, which could adjust values of observed interpretations. Alterations in visceral organ weights have been accepted to denote distortions in physiochemical integrity and may suggest underlying pathologic state consequent upon exposure of biologic systems to toxicants.[43–47] For instance, Chua *et al.,*[48] reported changes in body and organ weights as basis for toxicological evaluation of dried kacangma *(Leonurus sibiricus)* in rats. From another study, Ahmed *et al.,*[5] applied the measurement of visceral organ weight (pancreas and liver weights) as a reliable diagnostic tool for ascertaining therapeutic performance of methanolic leaf and callus extracts of *Gymnema sylvestre* in diabetic Wistar rats. The increase in pancreas and liver weights in groups H-ETH-E and H-AQ-E conformed to the reports by Ahmed *et al.,.*[5] However, the paradoxical increase in liver weight of the untreated group (C-H) may well denote liver disarrangement in the form of fatty infiltration with attendant enlargement of the liver and tissue hyperplasia as result of absence of glycemic control in the experimental rats[42,49,50] Likewise, the significant increases in pancreas of the experimental groups C-H and H-ETH-E were more of toxicological implications than therapeutic considerations.

The present report showed that rats administered with leaf extract of *V. album* as well as those without glycemic control did not exhibit significant ($p > 0.05$) alterations in kidney weights. However, the findings here appear to contradict the reports of Zafar and Naqvi,[42] in which they noted that streptozotocin treated animals without glycemic control exhibited increased kidney weight (hypertrophy). Nevertheless, these contradictory reports could be reconciled when the difference in durations of the two experiments are taken into consideration. According to Sharma and Ziyadeh,[51] overexpression of transforming growth factor (TGF)–β1 is connected with the development of renal hypertrophy in type 1 DM. In addition, Zafar and Naqvi,[42] mentioned the implication of growth hormone (GF) and insulin-like growth factors (IGFS) in renal hypertrophy. Thus, the relatively extended experimental time (8 weeks) of Zafar and Naqvi,[42] investigations allowed for the manifestation of renal hypertrophy compared short-term (2 weeks) investigation of the present study. Ren *et al.*,[52] have previously reported a

marked enlargement and increase in kidney weight in streptozotocin-induced diabetic rats from 8 to 16 weeks. Therefore, the non-significant increase in kidney weight, especially in group C-H, may be due to the limits of the experimental time to allow for the development of renal hypertrophy.

In a related study, James *et al.,*[53] showed that chemically induced splenic injury engendered significant ($p < 0.05$) increase in spleen weight in animal model. They further noted that CCl_4 induced injury of the splenic sinusoids was reversed following the administration of leaf extract of *Vitex doniana* with the spleen weight of the treated animals comparable to the control. The alterations of spleen weights of the various experimental groups (Table 1) were indications of splenic disarrangement that tended towards restoration to normal physiologic weight, following the administration leaf extract of *V. album,* within the constraint of limit of the experimental time. Likewise, the improvements in pancreas weights of diabetic rats subsequent to administration of the leaf extracts suggested extra-pancreatic mechanisms of action of *V. album* as previously posited elsewhere.[11,54,55]

It is worthwhile to note that within the ambit of alterations in visceral organ weight as a basis for toxicologic assessment of *V. album* leaf extracts, indicators showed that administration of the leaf extracts at the present dose and form did not affect the heart and kidneys of the experimental rats. Furthermore, although both aqueous and ethanolic leaf extracts of *V. album* exhibited glycemic control in the experimental rats, the extracts did not possess the capacity to restore full therapeutic benefits within the limits of experimental time. Accordingly, the therapeutic thresholds of the leaf extracts, exemplified by levels of %FBG and improvements in *b/w*s of corresponding experimental groups compared to the group C-N, might be in connection with pharmacognostic dynamics coupled with toxicological outcomes of the administered leaf extracts.

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