Original Research Article

Ethylacetate fraction of *Anthocleista vogelii* Planch demonstrates antiobesity activities in preclinical models

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Abstract

Purpose: To assess the anti-obesity effect of liquid chromatography mass spectrometry (LCMS) profiled ethylacetate fraction (EF) of Anthocleista vogelii Planch on pancreatic lipase activity in vitro, and on obesity-related hormones in high-fat diet (HFD)-induced obese rats.

Methods: Chromatographic analysis of EF to identify bioactive compounds was performed using LCMS electrospray ionization mass spectrometry (ESI-MS) positive mode. Thirty Sprague–Dawley rats were divided into 5 groups (n = 6). Group 1 was fed normal pellet diet, while groups 2 - 5 were fed high-fat diet (HFD) for 14 weeks. The rats were treated for 4 weeks from week 10 with 125 mg/kg of EF (group 3), 250 mg/kg of EF (group 4) or 100 mg/kg of orlistat (group 5).

Results: Seven alkaloids were identified in EF, namely, 10-hydroxycamtothecin, moschamindole, camptothecin, moschamine, N6-cis-p-coumaroylserotonin, sinomenine and desacetylcolchicine. The EF of A. vogelii exhibited inhibitory activity against pancreatic lipase with half-maximal inhibitory concentration (IC_{50}) of 8.76 ± 0.110 μg/mL. Rats treated with EF (125 and 250 mg/kg) of A. vogelii showed significantly (p < 0.05) decreased feed intake, body weight, leptin and insulin, when compared to HFD controls. Cortisol, serotonin and noradrenaline were significantly (p < 0.05) increased, but changes in thyroid hormones levels in EF-treated rats were not significant (p > 0.05) when compared to HFD controls.

Conclusion: The EF of A. vogelii demonstrate anti-obesity activities by inhibiting pancreatic lipase, elevating serotonin and noradrenaline, and increasing leptin sensitivity, leading consequently to decreased body weight of rats. However, the clinical use of EF of A. vogelii as an antiobesity herbal remedy requires further studies on its mechanisms of action.

Keywords: Obesity, Anthocleista vogelii, Lipase, High-fat diet, Cortisol, Serotonin, Noradrenaline, Leptin, Insulin

INTRODUCTION

Obesity can be defined as an increase in body weight beyond the limits of physical need, resulting from excessive accumulation of fat [1]. It is a chronic condition that is better prevented than cured, because it is difficult to treat. When prevention is unsuccessful, medicinal treatment

of obesity becomes vital to prevent the development of hypertension, diabetes or heart diseases which are usually associated with obesity [2]. Today, many individuals are sincerely concerned about their body weight due to the increasing sensitization, the health risks or social stigmatization associated with obesity. In the treatment and management of obesity, drugs such as sibutramine, phentermine, exenatide, pramlintide, metreleptin and orlistat (pancreatic lipase inhibitor) have been used over the years, but these drugs have their side effects [3]. While sibutramine has been withdrawn, orlistat remains an over-the-counter drug in many countries, however, orlistat has side effects like flatulence and oily stools [3].

In the search for new antiobesity drugs, researchers are continually investigating lipase inhibitory activity, and regulation of neurotransmitters and endocrine hormones, as possible therapeutic targets. Neurotransmitters like serotonin (5HT), noradrenalin (NA) and dopamine (DA), and hormones like leptin, insulin, triiodothyronine (T3), thyroxine (T4), thyroid-stimulating hormone (TSH) and cortisol are some of the modulators that have been the focus of obesity research [2,3,4].

Research into antiobesity agents from plants is ongoing in different parts of the world. *Anthocleista vogelii* is one plant that has received recent attention. The ethnobotany and pharmacological properties of *A. vogelii* have been well reviewed [5]. This study sought to evaluate the weight, neurotransmitter and endocrine status of diet induced obese Sprague-Dawley rats treated with ethylacetate fraction (EF) from *Anthocleista vogelii* root bark, and the lipase inhibitory activity *in vitro* of the EF.

EXPERIMENTAL

Plant material

The fresh parts of *A. vogelii* root bark was obtained from Ovuakali, Umuekwune community, Ngor-okpala, Imo State, Nigeria with authorization of Ngor-okpala Local Government. The plant identification and authentication was done by Dr JO Ihuma, Biological Sciences Department, Bingham University, Nasarawa State, Nigeria. The plant was allocated the voucher number, GA134-7421.

Preparation of ethyl acetate fraction

The EF was prepared by macerating 3 kg of dried powdered root bark of *A. vogelii* in methanol, and the concentrated methanol extract was defatted by extracting with n-hexane. The defatted methanol extract marc was extracted and partitioned with n-butanol saturated with water (3x500 mL). The aqueous extract was partitioned 3 times with 500 mL of ethyl acetate using a separating funnel. The ethyl acetate partitions were pooled together and concentrated using a rotary evaporator to obtain EF.

LC-MS/MS analysis

The chromatographic separations of diluted EF were executed on Agilent 1290 Infinity LC system attached to Agilent 6520 Accurate-Mass Q-TOF mass spectrometer on ESI positive mode. The LC-ESI-MS parameters and experiment were performed as previously described by Anyanwu *et al* [6]. The MS parameters were as follows: source voltage was 3500V, the Fragmentor voltage and Skimmer were 125V and 65V respectively and OCT 1 RF Vpp was 750V. The drying gas was set at 10 L/min and gas temperature at 300 $^{\circ}$C. Analyses were carried out on full scan mode, 100 – 3200 mass range (m/z), and processing of acquired data was done with Agilent MassHunter Qualitative Analysis B.05.00.

Pancreatic lipase inhibitory activity

The method used to determine the inhibitory pancreatic lipase activity for EF was as described by Kim *et al* [7] with slight modifications. Twenty microlitre of either EF or orlistat at different concentrations (2.35, 4.69, 9.38, 18.75, 37.5, 75, 150, and 300 µg/mL) was added to 164 µL of assay buffer and 6 µL pancreatic lipase solution in each well. After incubation for 10 min at 37 °C, 10 µL of 10 mM p-NPB (p-nitrophenyl butyrate) in assay buffer was added and incubated at 37°C for 15 min. Absorbance readings were taken at 405 nm using a microplate reader. EF inhibition of pancreatic lipase activity (L) was calculated as in Eq 1.

$$L\ (\%) = 100 - \{(B\text{-}b/A\text{-}a)\}100 \ldots\ldots\ldots (1)$$

where B = activity with inhibitor, A = activity without inhibitor, b = negative control with inhibitor, and a = negative control without inhibitor.

Animals

Thirty male Sprague–Dawley rats (150 - 170 g) were purchased from the National Institute of Health, Islamabad, Pakistan and housed in cages at the Animal House, Department of Pharmacy, COMSATS Institute of Information Technology, Abbottabad, Pakistan.

Acclimatization of the rats under controlled temperature (23 ± 2°C) and 12:12 h light/dark cycle with water and food *ad libitum* took 2 weeks. Experimental procedures followed European Community guidelines [8] for animal handling and ethical approval (no. PHM-0024/EC/M-4-5.15) was obtained from Research Ethics Committee of Pharmacy Department, CUI, Abbottabad.

Animal grouping and induction of obesity

The rats were separated into 5 groups of 6 rats per group. Normal pellet diet (NPD) was given to rats in group 1, while groups 2-5 were fed a high fat diet (HFD) for 14 weeks; water was given *ad libitum*. The HFD was composed of 60 % fat content as described by Onyeneke and Anyanwu [9].

Obesity was induced in 24 rats by feeding with the HFD for 10 weeks while the other 6 rats were fed on the NPD. Treatment with 125 mg/kg of EF (Group 3), 250 mg/kg of EF (Group 4) and 100 mg/kg of orlistat (Group 5) began after the 10th week and lasted for 4 weeks, but the HFD obese control (Group 2) and NPD control (Group 1) received only normal saline. Doses were selected based on previous unpublished studies. The fraction or standard drug was dissolved in normal saline and orally administered to the animals using a gavage tube.

Blood samples

At the close of the treatment period, the rats were fasted for 13 ± 1 h, then anesthetized with isoflurane before blood samples were collected from the rats *via* cardiac puncture. The blood samples were collected in tubes void of anticoagulant, and after coagulation, they were placed in a centrifuge then spun at 3500 rpm for 15 min at 25 ^{o}C. The serum was separated into new tubes using micro-pipette and preserved at -20 °C.

Brain tissues

The brain was removed from the skull of the rats and the hypothalamus excised. The brain tissue was rinsed with 1X phosphate buffered saline (PBS), homogenized in 0.2 N perchloric acid ($HClO_4$), and centrifuged at 10,000 rpm for 5 min at 2 ^{o}C. The supernatant was collected and refrigerated at -20 ^{o}C.

Food intake, body weight and fat mass

The food intake was recorded daily using a 2-decimal-place balance (g/day/cage). The body weight of the rats were recorded weekly in grams (g). After blood sample collection as earlier described, the anesthetized rats were dissected from the abdomen, and the white adipose tissue (WAT) which included the epididymal, visceral and retroperitoneal pads, was harvested from each rat. The WAT was placed on filter paper and weighed in grams.

Evaluation of biochemical parameters

Total cholesterol was determined by the enzymatic endpoint method as described by Trinder [10]. Triglyceride was determined by GPO-PAP method as described by Tietz [11]. HDL-Cholesterol was determined by CHOD-PAP method as described by Tietz [12]. LDL-Cholesterol assay was based on manufacturer's protocol (Spectrum Diagnostics, Egypt). VLDL-Cholesterol was calculated as triglycerides value multiplied by 0.2, and expressed in mg/dL.

Serum was used to determine the concentrations of total T3, T4, TSH and cortisol by MicroLISA Enzyme Immunoassay test kits (Amgenix International Inc., USA). Supernatants from brain samples were used to assay for serotonin (5-HT) (EIA Serotonin kit, Immunotech, France), noradrenaline (NA) and dopamine (DA) (NA and DA ELISA kit, Cusabio, China) by the enzyme immunoassay technique according to the manufacturer's manual. All blood glucose measurements were performed on blood isolated from the tail using an Accu-Chek glucometer (Roche, Germany). The levels of serum insulin and leptin were determined based on the sandwich ELISA principle (DRG Leptin and Insulin ELISA kits, USA).

Histopathological studies

A part of the liver was excised, washed with water and placed in 10% neutral buffered formalin for histopathology examination. Sections of the liver were prepared and stained with haematoxylin and eosin following fixation. Permanent mounts were examined by light microscopy and the results obtained from treated rats were compared with controls [13].

Statistical analysis

The results of the experiments were arranged as mean ± SEM (standard error of mean). Data collected were analyzed on Graphpad Prism 6 using one-way ANOVA, followed by Tukey multiple comparison test to ascertain the significant differences amongst groups. $P < 0.05$ was set as statistically significant.

RESULTS

Phytochemical composition of EF

The classes of phytochemicals included in the EF were benzoic acids, amino acids, organic acids and derivatives, lipids, terpenoids, alkaloids, imides, flavonoids (Supplementary information). Alkaloids were more in number than other classes of phytochemicals found in EF of *A. vogelii*, and these include: 10-Hydroxycamtothecin (13), moschamindole (15), camptothecin (16), moschamine (17), N6-cis-p-coumaroylserotonin (18), sinomenine (19,20) and desacetylcolchicine (24) (Figure 1, Table 1).
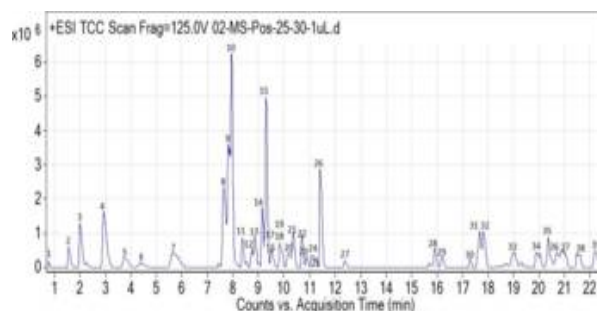

Figure 1: Total compound chromatogram (TCC) of LC-ESI/MS analysis of EF of *A. vogelii*

Table 1: Report of compounds identified in EF of *A. vogelii*

Peak	RT	Mass	Name	Formula	Class
1	0.77	165.08	Benzocaine	C9 H11 N O2	Benzoic acid esters
2	1.57	179.06	p-Acetaminobenzoic acid	C9 H9 N O3	Amidobenzoic acid
3	2.017	163.06	p-Acetaminobenzaldehyde	C9 H9 N O2	Amidobenzoic acid
4	2.951	193.07	Phenylacetylglycine	C10 H11 N O3	n-acyl-alpha amino acids
5	3.766	164.08	gamma-Thujaplicin	C10 H12 O2	Tropolones
6	4.403	170.09	Furfural diethyl acetal	C9 H14 O3	Furans
7	5.724	177.08	2-Propenyl 2-aminobenzoate	C10 H11 N O2	Benzoic acid esters
8	7.627	207.09	Phenylpropionylglycine	C11 H13 N O3	n-acyl-alpha amino acids
9	7.815	236.12	Ala Phe	C12 H16 N2 O3	Dipeptide
10	7.95	366.12	N-Caffeoyltryptophan	C20 H18 N2 O5	n-acyl-alpha amino acids
11	8.365	164.08	gamma-Thujaplicin	C10 H12 O2	Tropolones
12	8.725	196.07	Xanthoxylin	C10 H12 O4	Alkyl-phenylketones
13	9.011	364.11	10-Hydroxycamtothecin	C20 H16 N2 O5	Quinolone alkaloid
14	9.294	196.07	Phlorisobutyrophenone	C10 H12 O4	Phenol
15	9.348	350.13	Moschamindole	C20 H18 N2 O4	Alkaloid
16	9.496	348.11	Camptothecin	C20 H16 N2 O4	Alkaloid
17	9.546	352.14	Moschamine	C20 H20 N2 O4	Alkaloid
18	9.621	322.13	N6-cis-p-Coumaroylserotonin	C19 H18 N2 O3	Alkaloid
19	9.927	329.16	Sinomenine	C19 H23 N O4	Morphinans/ Opiate alkaloids
20	10.217	329.16	Sinomenine	C19 H23 N O4	Morphinans/ Opiate alkaloids
21	10.359	298.13	Unknown	C17 H18 N2 O3	
22	10.702	209.10	Tyr-OEt	C11 H15 N O3	Amino acids, aromatic
23	10.882	328.14	Tyr Phe	C18 H20 N2 O4	Dipeptides
24	11.082	357.16	Desacetylcolchicine	C20 H23 N O5	Alkaloids
25	11.236	462.15	Exoticin	C23 H26 O10	Flavones and flavonols
26	11.421	324.15	p-Hydroxyphenylbutazone	C19 H20 N2 O3	Phenylbutazone
27	12.372	273.27	C16 Sphinganine	C16 H35 N O2	Ceramide/ 1,2-aminoalcohols
28	15.906	324.19	Dinor-PGE2	C18 H28 O5	Prostaglandins
29	16.187	324.19	Dinor-PGE2	C18 H28 O5	Prostaglandins
30	17.285	290.19	Unknown	C18 H26 O3	
31	17.657	278.15	Emmotin A	C16 H22 O4	Sesquiterpenoids
32	17.821	180.07	Protionamide	C9 H12 N2 S	Isonicotinic acid
33	18.974	437.37	Unknown	C24 H47 N5 O2	
34	19.956	281.27	Oleamide	C18 H35 N O	Fatty amides
35	20.475	378.28	9-deoxy-9-methylene-16,16-dimethyl -PGE2	C23 H38 O4	Prostaglandins
36	20.73	342.28	Eicosanedioic acid	C20 H38 O4	Alpha,omega-dicarboxylic acid
37	21.027	364.30	24-Nor-5β-cholane-3α,7α,23-triol	C23 H40 O3	Bile acids and derivatives
38	21.455	390.28	3α,12α-Dihydroxy-5β-chol-8(14)-en-24-oic Acid	C24 H38 O4	Bile acid
39	22.19	586.29	Unknown	C28 H46 N2 O9 S	

Inhibitory activity of EF against pancreatic lipase

The EF of *A. vogelii* displayed inhibitory activity against pancreatic lipase with IC_{50} value of 8.76 ± 0.110 µg/mL (Figure 2), although not as good as Orlistat (IC_{50} = 0.73 ± 0.015 µg/mL).
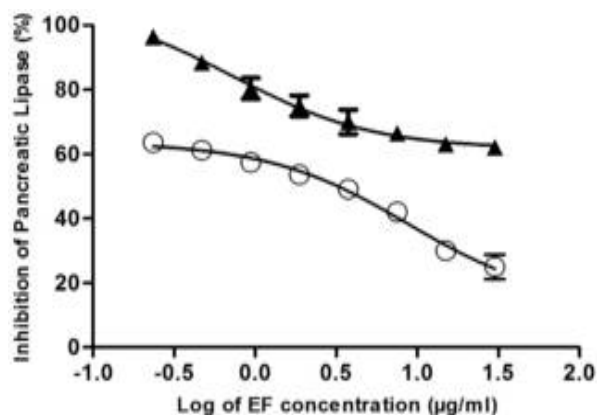

Figure 2: Pancreatic lipase inhibitory activity of EF of *A. vogelii* and Orlistat. ▲ = EF of *A. vogelii*; and ○ = Orlistat. The values are expressed as the mean ± SEM (n = 3)

Feed intake, body weight, fat mass and lipid profile

The group given only HFD had significantly ($p < 0.05$) increased body weight of rats when compared to the normal control (Table 2). Rats treated with EF (125 and 250 mg/kg) of *A. vogelii* displayed significant (p < 0.05) decrease in the feed intake and body weight compared to both the normal control and HFD control.

The WAT of the HFD control was significantly ($p < 0.05$) higher when compared to the normal control (Table 2). However, EF (125 and 250 mg/kg) of *A. vogelii* significantly (p < 0.05) decreased WAT when compared to the HFD and normal control. The HFD control group revealed significant ($p < 0.05$) increases in TG, TC, LDL-C and VLDL-C, but a significant (p < 0.05) decrease in HDL-C when compared to the normal control (Table 2). The EF (125 and 250 mg/kg) of *A. vogelii* significantly ($p < 0.05$) decreased TG, TC, LDL-C and VLDL-C but increased significantly ($p < 0.05$) the HDL-C compared to the HFD control. Also, in comparison with the normal control, EF (125 and 250 mg/kg) of *A. vogelii* reduced significantly ($p < 0.05$) the TG, TC and VLDL-C only.

Glucose, neurotransmitters and endocrine status of obese rats

The HFD control group showed a significant ($p < 0.05$) increase in TSH, a decrease (p < 0.05) in T4 and no significant ($p > 0.05$) changes in T3 and cortisol levels when compared to the normal control (Table 3). The group treated with EF revealed a significant ($p < 0.05$) decrease in T4, a significant ($p < 0.05$) elevation in cortisol and non-significant ($p < 0.05$) changes in T3 and TSH levels when compared to the HFD control.

The rats in the HFD control group had significantly (p < 0.05) reduced 5HT and NA levels but significantly (p < 0.05) elevated DA compared to the normal control (Table 3). The 125 and 250 mg/kg EF revealed significant (p < 0.05) increases in 5HT and NA, but significant (p < 0.05) decrease in DA compared to the HFD control. There were significant increases in leptin, insulin and glucose levels of the HFD fed rats when compared to the normal control (Table 3). The animals treated with 125 and 250 mg/kg EF showed significant (p < 0.05) decreases in leptin, insulin and glucose levels when compared to HFD control (p < 0.05).

Table 2: Effect of EF on food intake, body weight, WAT and lipid profile in obese rats

Parameter	NPD control	HFD control	Av EF (125 mg/kg)	Av EF (250 mg/kg)	Orlistat (100 mg/kg)
	Group 1	*Group 2*	*Group 3*	*Group 4*	*Group 5*
Food intake (g)	30.65 ± 0.90[a]	29.67 ± 1.86[a]	17.46 ± 2.41[bc]	15.79 ± 1.54[c]	29.66 ± 0.67[a]
Body weight (g)	272.67 ± 7.13[bc]	312.00 ± 12.49[a]	263.00 ± 9.71[c]	253.00 ± 10.21[c]	275.33 ± 4.67[bc]
WAT (g)	12.80 ± 0.27[b]	17.96 ± 0.84[a]	4.96 ± 1.77[c]	4.32 ± 1.52[c]	12.74 ± 0.86[b]
TG (mg/dL)	117.38 ± 4.88[b]	133.05 ± 2.94[a]	92.89 ± 2.27[cd]	89.89 ± 1.85[d]	92.98 ± 1.07[cd]
TC (mg/dL)	100.49 ± 1.17[d]	130.75 ± 2.25[a]	108.16 ± 4.30[bc]	105.16 ± 4.40[cd]	115.05 ± 1.29[bc]
LDL-C (mg/dL)	42.20 ± 2.94[c]	104.93 ± 2.24[a]	84.54 ± 4.08[b]	81.54 ± 3.96[b]	79.40 ± 2.81[b]
HDL-C (mg/dL)	38.94 ± 0.97[c]	37.02 ± 0.88[c]	58.71 ± 3.13[ab]	61.61 ± 3.10[a]	42.29 ± 1.19[c]
VLDL-C (mg/dL)	23.48 ± 0.98[b]	26.61 ± 0.59[a]	18.58 ± 0.45[c]	17.98 ± 0.37[c]	18.60 ± 0.21[c]

Values are presented as mean ± SEM. Means in the same rows not sharing common letter(s) are significantly different ($p < 0.05$).

Table 3: Effect of EF on neurotransmitters, hormones and glucose concentrations in obese rats

Parameter	NPD control	HFD control	Av EF (125 mg/kg)	Av EF (250 mg/kg)	Orlistat (100 mg/kg)
	Group 1	*Group 2*	*Group 3*	*Group 4*	*Group 5*
T3 (µg/dL)	1.56 ± 0.25[b]	1.49 ± 0.06[b]	1.49 ± 0.05[b]	1.40 ± 0.08[b]	0.63 ± 0.14[c]
TSH (µg/dL)	0.12 ± 0.00[d]	0.20 ± 0.00[a]	0.20 ± 0.01[a]	0.17 ± 0.02[bc]	0.19 ± 0.01[ab]
Cortisol (µg/dL)	0.91 ± 0.81[c]	1.15 ± 1.10[c]	8.42 ± 0.88[a]	7.42 ± 0.90[a]	5.66 ± 0.61[ab]
5HT (ng/g)	11.51 ± 0.53[b]	8.75 ± 1.37[c]	15.24 ± 0.75[ab]	16.58 ± 0.58[a]	10.98 ± 0.85[b]
DA (ng/mL)	1.35 ± 0.03[b]	7.63 ± 0.79[a]	0.43 ± 0.09[c]	0.45 ± 0.07[c]	0.33 ± 0.02[c]
NA (pg/mL)	19.34 ± 2.78[b]	15.42 ± 1.80[c]	68.82 ± 3.86[a]	70.48 ± 2.36[a]	79.47 ± 9.55[a]
Leptin (ng/mL)	0.09 ± 0.01[c]	0.60 ± 0.07[a]	0.43 ± 0.04[b]	0.33 ± 0.03[b]	0.64 ± 0.04[a]
Insulin (µIU/mL)	1.75 ± 0.18[c]	3.79 ± 0.43[a]	1.93 ± 0.11[bc]	1.73 ± 0.04[c]	2.37 ± 0.12[b]
Glucose (mmol/L)	11.73 ± 0.41[b]	16.50 ± 0.50[a]	12.07 ± 0.11[b]	11.52 ± 0.43[b]	11.51 ± 0.49[b]

Values are presented as mean ± SEM. Different letter(s) on the mean values in the same row indicates significant difference ($p < 0.05$)

Histopathological features of liver

Photomicrographs of the liver revealed that the HFD control groups had large fat deposits at focal areas and moderate inflammation (Figure 3). Rats treated with 125 mg/kg EF showed slight distortion in the liver structure with numerous microvascular fat deposits. However, rats treated with 250 mg/kg EF showed normal liver morphology without structural distortions or fat deposits.
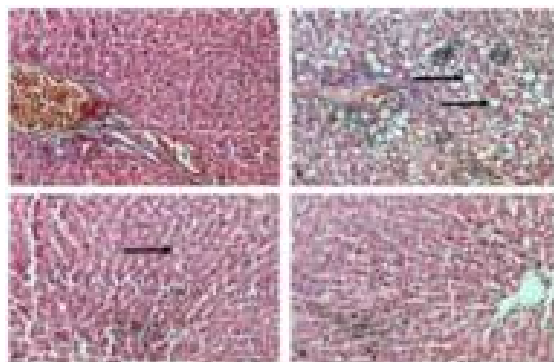

Figure 3: Photomicrograph of the liver of rats treated with EF of *A. vogelii*. **Note:** Light microscope images of liver tissues stained with hematoxylin and eosin were of X20 magnification. A- Normal control; B- HFD control and (→) indicates presence macrovascular fat deposit; C- 125 mg/kg EF and (→) indicates microvascular fat deposit; and D- 250 mg/kg EF shows reversal of fat deposit

DISCUSSION

The compounds in EF listed on Table 1 have not been reported for possible anti-obesity activities except benzocaine, phenylacetylglycine and moschamine. Benzocaine in combination with phenylpropanolamine has been shown to have weight loss effects, although benzocaine as a single drug was ineffective based on a single randomized clinical trial [14]. Moschamine has been reported to possess serotonergic inhibitory activities [15], while phenylacetylglycine has been shown to be involved in obesity however, its effect is not yet clear [16].

Pancreatic lipase, secreted from the pancreas facilitates the absorption of triglycerides in the small intestine from dietary fat, thus, inhibitors of pancreatic lipases function as antiobesity agents. Ethylacetate fraction from *A. vogelii* inhibited the activity of PLE suggesting its antiobesity potential, and this was evidenced in the reduced body weight and fat mass of animals that received EF of *A. vogelii.* Reduced absorption of ingested dietary fat leads to an overall reduced caloric absorption, thereby leading to weight loss [17].

According to de Graaf *et al* [18] appetite could be determined by measuring actual food intake (i.e., the amount of food consumed within a specific context). The EF of *A. vogelii* reduced the food intake of the animals, and by implication their appetite was reduced. The decrease in body weight and WAT of the animals could be partly attributed to the significant decrease in their food intake. Since increased fat mass can be directly correlated with obesity [19], the reduction in WAT observed in the treated rats is an indication of the antiobesity potential of EF. The findings that EF reduced the levels of serum TG, TC, LDL-C and VLDL-C is similar to an earlier study for ethanol extracts of *A. vogelii* root bark at a higher concentration of 500 mg/kg [20],

Research has shown that TSH, T3, and T4 are elevated in obese individuals when compared to those of normal weight [21]. As a result, weight reduction should result in a decline in T3, T4 concentrations, but not in TSH concentrations [21]. However, the EF of *A. vogelii* caused decrease in T4, with no changes in T3 or TSH levels. This indicated that EF had no discernable

impact on thyroid hormones in obese rats. The EF increased the levels of cortisol, which might indicate an adjustment to weight reduction in the animals as higher cortisol levels have been linked to weight loss [22].

The elevated levels of 5-HT and NA in the treated animals indicated that EF inhibited 5-HT and NA reuptake thereby increasing the levels of 5-HT and NA in the hypothalamus and consequently suppressing food consumption and reducing body weight. Moschamine, which was identified in EF, has been reported to have serotoninergic inhibitory activity [15]. The EF fractions of *A. vogelii* decreased dopamine levels, which indicate that the fractions might cause increased sensitivity of dopamine in the animals, as dopamine is associated with meal size and satiety [23]. Obesity is characterized by increase in serum glucose, insulin and leptin values [24]. These features were observed in the animals fed with HFD without treatment. The EF of *A. vogelii* effectively decreased levels of leptin, insulin and glucose, thereby reversing the obesity state of the animals. Nonalcoholic fatty liver disease (NAFLD), a medical condition of excess fat deposit in the liver is a common feature of obesity [25]. The 250 mg/kg EF of *A. vogelii* induced a complete reversal of the fatty liver condition of the animals.

CONCLUSION

Overall, the EF of *A. vogelii* exerts significant weight loss effects on rats due to the inhibition of pancreatic lipase and/or increasing levels of serotonin (which suppresses appetite) and noradrenaline (which increases calorie dissipation). The presence of moschamine probably contributes to the plant's serotoninergic inhibitory activity thereby decreasing food intake. Clinical studies are required to determine if EF may also cause reduction in the body weight of humans.

Supporting information

The total ion chromatogram (TIC) scan, total compound chromatogram (TCC) and tabulated data for all compounds in EF of *A. vogelii* obtained from LCMS/MS positive mode are available as supporting information.

DECLARATIONS

Acknowledgement

Conflict of interest

The authors declare that no conflict of interest is associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors". This study was conceived by Dr. Gabriel and Prof. Onyeneke, the design and supervision was done with contributions from Prof. Nisar-ur-Rahman and Dr. Khalid. The experimental work was done by Dr. Gabriel and Hafiz Misbah, while the LCMS/MS analysis was by Dr. Gabriel and Mr. Khan. Biochemical analysis was done with the support of Prof. Iqbal (Director of CADR) and Abida Ejaz. Dr. Gabriel analyzed the data and wrote the manuscript, however all authors read, edited and approved the manuscript for publication.

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