Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Angiopteris helferiana, a Fern with Great Potential Medicinal Value: Antiadipogenic, Anti-Inflammatory, and Anti-Diabetic Activity

Ramakanta Lamichhane, Prakash Raj Pandeya, Kyung-Hee Lee, Se-Gun Kim[1], Dhan Raj Kandel[2], Hyun-Ju Jung

Department of Oriental Pharmacy, College of Pharmacy, Wonkwang-Oriental Medicines Research Institute, Wonkwang University, Iksan, [1]Department of Agricultural Biology, National Academy of Agricultural Science, Rural Development Administration, Wanju, Republic of Korea, [2]Department of Plant Resources, National Herbarium and Plant Laboratories, Lalitpur, Nepal

Submitted: 09-08-2018 Revised: 27-08-2018 Published: 16-05-2019

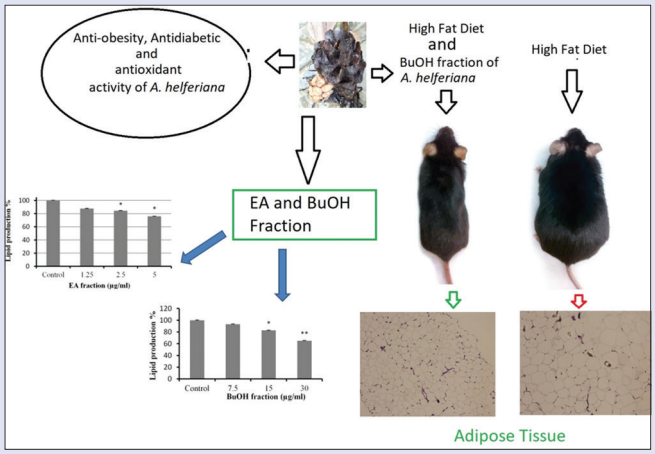
ABSTRACT

Background: *Angiopteris helferiana* is used as a traditional medicine in some parts of Nepal and South-Asian countries. It is also used in traditional Chinese medicine system. **Objective:** In this study, we evaluated its anti-oxidant, anti-inflammatory, anti-obesity, and antidiabetic activity in cell and animal mode. **Materials and Methods:** The rhizome of *A. helferiana* was extracted with methanol and fractionated to give dichloromethane, ethyl acetate (EA), butanol (BuOH), and water fractions. The residue was again re-extracted with water to give water extract. The anti-oxidant property was measured using 1, 1-diphenyl-2-picryl-hydrazyl assay. The extract and fractions were evaluated for the α-glycosidase inhibition activity. The antiadipogenic and anti-inflammatory activity was studied in 3T3-L1–L1 and RAW 264.7 cells, respectively. The inhibition of expression of adipogenic markers peroxisome proliferator-activated receptor gamma (PPARγ), CCAAT enhancer binding protein alpha (C/EBPα) was studied in 3T3-L1 cells. The anti-obesity and antidiabetic study was done in high-fat diet mice model. **Results:** The EA, BuOH fraction, and water extract showed good anti-oxidant and α-glycosidase inhibition activity. EA fraction showed good anti-inflammatory activity. EA and BuOH fraction showed good antiadipogenic activity, reducing significantly, the lipid and adipogenic markers (PPARγ, C/EBPα) in 3T3-L1 cells. The BuOH fraction at 300 mg/kg/day dose showed good anti-obesity and antidiabetic activity in the *in-vivo* study after the evaluation of body weight, blood lipid, blood glucose, and lipid accumulation in adipose tissue and liver. **Conclusion:** Overall, *A. helferiana* is a good source of anti-oxidative, anti-inflammatory, anti-obesity, and antidiabetic component.

Key words: *Angiopteris helferiana,* anti-adipogenic, anti-inflammatory, antioxidant, obesity

SUMMARY

- *Angiopteris helferiana*, a fern with traditional medicinal value was studied for various biological activities through various *in-vitro* and *in-vivo* studies. The DPPH assay revealed the plant with potent anti-oxidant activity. The EA fraction of the plant showed good anti-inflammatory activity in RAW cells experiment. The strong lipid inhibiting activity of the plant was established according to the results form cell and animal experiment. In cell (3T3-L1), the EA and BuOH fraction of the plant strongly inhibited the adipogenic markers. In animal study, the results showed the reduction of lipid in both blood and adipose tissue after the oral feeding of the BuOH fraction of the plant which was evaluated from biochemical analysis and histology. There was also reduction of blood glucose level in the high-fat diet fed mice after the administration of the plant sample. Overall the plant showed good antioxidant, anti-inflammatory, anti-adipogenic, and glucose-lowering activity. Hence, the plant has good potential for the herbal medicine for various ailments.



Abbreviations used: PPARγ: Peroxisome proliferator-activated receptor gamma; C/EBPα: CCAAT enhancer binding protein alpha; *A. helferiana: Angiopteris helferiana.*

Correspondence:

Dr. Hyun-Ju Jung,
Department of Oriental Pharmacy, Wonkwang University, Iksan 54538, Republic of Korea.
E-mail: hyun104@wku.ac.kr
DOI: 10.4103/pm.pm_430_18

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

The use of plants for medicinal purpose dates back to a long time. Due to the toxicity and side effects of allopathic medicines, the research on plant-based medicine for different diseases is growing rapidly. Several research findings have established the medicinal properties of many plants. The various biological activates that the plants possess have played a great role in the treatment of numerous human diseases. Like the angiosperms, the various biological activities of ferns have also been reported, such as antibacterial, antiosteoporosis, anti-Alzheimer's disease activity, hypolipidemic, and hypoglycemic activities.[1] In the traditional Chinese medicine system and Ayurveda (traditional medicine system

Cite this article as: Lamichhane R, Pandeya PR, Lee KH, Kim SG, Kandel DR, Jung HJ. *Angiopteris helferiana*, a fern with great potential medicinal value: Antiadipogenic, anti-inflammatory, and anti-diabetic activity. Phcog Mag 2019;15:423-32.

of India and Nepal), hundreds of ferns have been mentioned with their medicinal values.[2,3] The present study was carried out to find biological activates of a fern and establish its medicinal properties.

Angiopteris helferiana is a fern belonging to *Marattiaceae* family. It is a gigantic fleshy plant with massive terrestrial bases due to erect, large-sized rhizome, 25–30 cm × 20–25 cm, densely covered with dark-brown to black hairs. It consists of long and branch-like smooth stipes consisting of huge bipinnate leaves with long, narrowly oblong pinnae bearing ultimate segments along their length and apically. The leaf consists of inframarginal sori on the ventral surface. *A. helferiana* is distributed in moist forest and slopes in an altitude of 900–1400 m in China, India, Nepal, Sri Lanka, and S. E. Asia.[4-6]

Some traditional medicinal values of *A. helferiana* have been reported in south Asian countries. In some parts of India, the rhizome of this plant is being used for scabies, indigestion, dysentery, and treatment of hair loss (in cattle) due to infection or injury.[7,8] In Nepal, the plant is used in muscle and bone pain/fatigue. *Angiopteris* species (Angiopteridaceae) have been widely used in traditional Chinese medicine to treat many diseases. Different research works on other species of *Angiopteris* have already been published. Different types of lactones (Angiopterlactones A and B), coumarin, b-sitosterol, and other compounds have been reported form other species of *Angiopteris*.[9]

In this study, the extract and fractions of rhizome and aerial part of *A. helferiana* were studied for the different biological activities *in vivo* and *in vitro*. The antioxidant activity was measured by different chemical tests. The antiadipogenic and anti-inflammatory activities were studied in 3T3-L1 and RAW 264.7 macrophage cells, respectively. The anti-diabetic activity was studied *in vitro* (α-Glucosidase inhibitory assay) and *in vivo* (mice). The anti-obesity activity of the plant was studied in high-fat diet (HF) animal model. The results showed that the plant *A. helferiana* possessed good antioxidant, antiadipogenic, anti-inflammatory, and anti-diabetic activity.

MATERIALS AND METHODS

Plant materials

The whole plants of *A. helferiana* were collected from a forest of Jamune-3, Tanahun, Gandaki, Nepal, during August and September 2015. After thorough washing, the plant materials were air dried under shade for 2–4 weeks. The rhizomes were separated from the plant and cut into small pieces. The plant was identified by Mr. Dhan Raj Kandel, Assistant researcher officer, National herbarium and plant laboratories, Godawari, Lalitpur, Nepal. It was identified as *A. helferiana* C. presl. A voucher specimen (2) has been deposited in the Herbarium of the National Herbarium and plant laboratories, Godawari, Lalitpur, Nepal.

Solvents reagents and chromatography supplies

Solvents including ethanol, methanol, ethyl acetate (EA), hexane, n-butanol (BuOH), and dichloromethane (DCM) were purchase form SK chemicals (Seongnam, Korea). 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) and dimethyl sulfoxide (DMSO) were purchased from Junsei chemicals (Junsei chemicals), sulfanilic acid, N-(1-napthyl) ethylenediamine dihydrochloride, folin ciocalteu reagent, and gallic acid were purchased from sigma aldrich and hydrogen peroxide from Daejung Chemicals Korea. Cell experiments: RAW 264.7 macrophage cells and 3T3-L1 preadipocyte cells were obtained from the American Type Culture Collection (Rockville, MD, USA). Dulbecco's modified eagle medium (DMEM), newborn calf serum (NCS), and fetal bovine serum (FBS) were obtained from Gibco, USA. 3-Isobutyl-1-methylxanthine (IBMX), dexamethasone, insulin, 10% formalin, isopropanol, and oil red O (ORO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Thiazolyl blue tetrazolium bromide (MTT), used to assess cell viability was purchased from Alfa Aesar, England. Qiazol lysis reagent for RNA isolation was purchased from Qiagen Sciences (Maryland, USA).

Extraction and fractionation

The rhizomes of plant *A. helferiana* were dried in shade for 2–4 weeks. The dried rhizomes (1.56 kg) were extracted with methanol using heat 40°C–50°C for 4–5 h. Methanol (MeOH) extract was dried in rotatory evaporator under vacuum and then suspended in water, followed by fractionation with hexane, DCM , EA, and BuOH, respectively. The hexane (Hex), DCM, EA (EA), BuOH, and water fractions obtained from the methanol extract were dried in rotatory evaporator under vacuum before storage for further experiment. The rhizome mass (residue) left after methanol extraction was again re-extracted with water using heat 40°C–50°C for 4–5 h. Thus, the obtained water extract was dried and stored. The air-dried aerial parts (stems and petiole) were also extracted with methanol to get methanol extract by the same procedure. It was dried in rotatory evaporator under vacuum and then fractionated with hexane, DCM, EA and BuOH. Figure 1 shows the extraction and fractionation process in brief.

Total flavonoid content test

Total flavonoid content in the methanol extract (rhizome and aerial parts) and fractions were determined as described by Arvouet-Grand *et al.* with some modifications. Stock quercetin solution was prepared in methanol.[10] In a 1.5 mL microtube, 250 µL of standard (quercetin) solution was mixed with 20 µL of 5% sodium nitrite ($NaNO_2$). To it, 300 µL of water and 75 µL of 10% aluminum chloride was added and mixed properly. Finally, 50 µL of 1 M NaOH was added and mixed properly. The whole solution was kept at room temperature for 10 min and then centrifuged. From the reaction mixture, 150 µL solution was taken and absorbance was measured at 510 nm in an ELISA reader. Absorbance corresponding to 0.25–62.5 µg for quercetin was recorded and calibration curve was prepared. Following the same procedure, absorbance for 2.5 mg of each

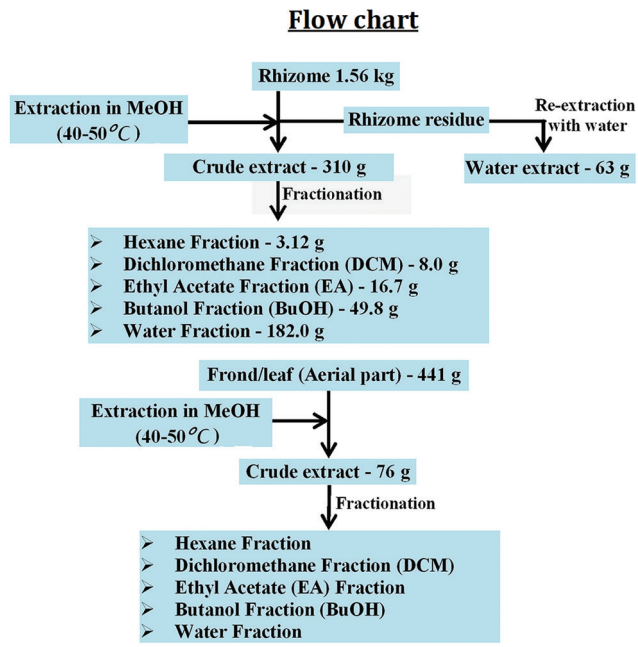


Figure 1: Flow chart of extraction and fractionation process for the rhizome and aerial parts of *Angiopteris helferiana*

extract or fraction of plant *A. helferiana* was measured and the total flavonoid content was calculated from the calibration plot and expressed as milligrams of quercetin equivalent (QE) per gram of extract or fraction.

Total polyphenol content test

The amount of total phenolics content was determined according to the Folin–Ciocalteu procedure.[11] The experimental calibration curve for the standard was prepared using gallic acid. In seven test tubes of volume 10 ml, 500 µL of 1N Folin-Ciocalteu reagent and 500 µL of ethanol were put. Then, aqueous solution of gallic acid (stock 2 mg/mL) was added to each test-tube in such a way that the amount of gallic acid in each test tube from 1 to 7 would be 0, 10, 20, 40, 80, 160, and 320 µg, respectively. The final volume in each test tube was adjusted to 3.5 mL by adding water and mixed thoroughly before leaving them for 5 min. Then, 500 µL of sodium carbonate (10%) was added to each solution and mixed thoroughly and kept in the dark for 90 min at room temperature. The absorbance was measured after 30 min at 760 nm in an ELISA reader. A standard curve was prepared for gallic acid by plotting the absorbance and concentration. The MeOH extract and other fractions (2 mg/mL) were conducted the same procedure, and the absorbance was measured. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram of extract, using the equation of the calibration curve and the obtained absorbance value.

1, 1-diphenyl-2-picryl-hydrazyl free radical scavenging antioxidant test

DPPH radical scavenging activity was measured using the method proposed by Hazra *et al.* with slight modification.[12] 200 µM DPPH solution was prepared in methanol. The sample (extract and fractions) was dissolved in DMSO. Ascorbic acid (in water) was prepared in different concentrations from 1.5 to 100 µg/mL. In 96-well plate, 100 µL of sample or standard solution was mixed with 100 µL of DPPH solution and incubated at 37°C for 30 min. The absorbance of the resulting solution was measured at 517 nm using an ELISA reader. In the blank for each sample, the DPPH solution was replaced by ethanol. The control contained blank DMSO or water and DDPH solution. The antioxidant activity was calculated as follows:

$$\text{Inhibition\%} = \frac{\left(A_{control} - \left[A_{sample} - A_{blank}\right]\right) \times 100}{A_{control}}$$

$A_{control}$ = absorbance of control, A_{sample} = absorbance of the sample with DPPH solution, A_{blank} = absorbance of the sample without DPPH solution.

The concentrations and respective percentage inhibition were plotted to get a curve, from which IC_{50} was calculated. The IC_{50} value was defined as the extract concentration required for inhibiting 50% DPPH activity under the assay conditions.

In vitro α-glucosidase inhibition assay

α-Glucosidase inhibitory assay was performed according to the method indicated by Mccue *et al.* with some modifications.[13] α-Glucosidase was diluted to 0.3 U/mL using 0.2 M phosphate buffer (pH 7.0). The substrate p-nitrophenyl glucopyranoside (pNPG) was prepared of concentration of 0.3 mM using the same buffer solution. The stock sample (extract and fractions dissolved in DMSO) were diluted with the buffer to the required concentrations. In a microplate 10 µL of buffer solution, 10 µL of samples, 10 µL of enzyme solution and 20 µL of pNPG solution were mixed and placed in incubator for 15 min at 37°C. The reaction was terminated by adding 150 µL of 0.2 M sodium carbonate solution. The final concentration of samples ranged from 2.5 to 100 µg/mL. In the blank, the enzyme solution was replaced by buffer solution. In the control, the sample was replaced by the buffer solution. The absorbance was measured with a microplate reader at 405 nm. Acarbose dissolved in buffer (200–40,000 µg/mL) was used as positive control. Each experiment was conducted in triplicate. The enzyme inhibitory rates of samples were calculated as follows in equation:

$$\text{Inhibition\%} = \frac{\left(A_{control} - \left[A_{sample} - A_{blank}\right]\right) \times 100}{A_{control}}$$

$A_{control}$ = absorbance of control, A_{sample} = absorbance of sample, A_{blank} = absorbance of the blank

The IC_{50} values of samples were calculated and reported as the mean ± standard deviation (SD) of three experiments.

Cell viability

Cell viability was determined colorimetrically using the MTT assay. Raw 264.7 cells were cultured overnight in 96-well plate at a density of 5×10^4 cells/200 µL in each well. 3T3-L1 pre-adipocytes were cultured in 48-well plate at a density of 2.5×10^4 cells/well. Raw 264.7 cells and 3T3-L1 cells were cultured in DMEM with 10% FBS and 10% NCS, respectively, at 37°C in 5% CO_2. After 24 h, medium was changed with 10% NCS or 10% FBS DMEM and the cells were treated with plant extracts and fractions at a final concentration of 5–100 µg/mL (in DMSO) for 2 days. The control received the same amount of DMSO. The final concentration of DMSO in the medium was not >1%. Then, MTT solution was added to the each well with final concentration 1 mg/mL and incubated for 4 h. Formazan formed by viable cells was dissolved with DMSO and absorbance was determined at 520 nm by ELISA reader.

Anti-inflammatory study on RAW 264.7 macrophage cells

Nitrate ions that accumulated in the cultural medium were measured as an indicator of NO production based on the Griess reaction. RAW 264.7 cells were plated in a 24 well plate at a density of 50×10^4 cells/500 µL in each well. After 24 h incubation (in a 95% air and 5% CO_2 humidified atmosphere at 37°C the cells were treated with different concentration of EA fraction. After 1 h, cells were treated with lipopolysaccharide (LPS) (1 µg/mL) in both extracts treated as well as untreated wells. Amounts of nitrite, a stable metabolite of NO, were measured using Griess reagent (1% sulfanilamide and 0.1% napthylethylenediamine dihydrochloride in 2.5% phosphoric acid). Briefly, 100 µL of cell culture medium was mixed with 100 µL of Griess reagent. Subsequently, the mixture was incubated at room temperature for 10 min and the absorbance at 540 nm was measured in a microplate reader. Fresh culture media was used as a blank. The quantity of nitrate was determined from a $NaNO_2$ standard curve.

Anti-adipogenic study on 3T3-L1 cells

3T3-L1 pre-adipocytes were cultured in DMEM with 10% bovine calf serum at 37°C in a humidified atmosphere of 5% CO_2. Two days after the 100% confluency (day 0), differentiation was induced by adding differentiation media (MDI), i.e., DMEM medium containing 0.5 mM IBMX, 1 µM Dexamethasone, 5 µg/mL insulin and 10% FBS. Two days after the induction of differentiation, on day 2, the culture medium was changed with DMEM supplemented with only 5 µg/mL insulin and 10% FBS. After that, the medium was changed on every 2 days (day 4, day 6, and day 8) with DMEM containing only 10% FBS. The cells were treated with samples (extract and fractions) on day 0 and day 2. The quantification of lipid production was done at day 10 by ORO staining.

Protein extraction and western blot analysis

3T3-L1 cells were differentiated as mentioned earlier with or without treating EA fraction (1.25, 2.5, and 5 μg/mL) and BuOH fraction (10, 20, and 30 μg/mL). On day 10, cells were collected using a cell scraper. Cell lysis was done by treating the cells with ice-cold RIPA buffer containing 25 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1% Nonidet P-40, 1% sodium deoxycholate, 0.1% SDS and a protease inhibitor cocktail (Sigma-Aldrich) for 30 min. All cell lysates were centrifuged at speed of 14,000 rpm for 20 min at 4°C to remove cell debris. Then, the protein concentration of each sample was calculated using a BCA protein assay kit (Pierce, Rockford, IL, USA). Cellular proteins (50 μg) were loaded and separated by 10% SDS-polyacrylamide gel electrophoresis. After that, the gels were transferred to nitrocellulose membranes at 150 mA for 1 h and were blocked with phosphate-buffered saline (PBS) containing 5% skim milk and 0.1% tween 20 for 2 h at room temperature. The blots were incubated with primary antibodies (1:1000 dilutions) overnight at 4°C followed by secondary antibody for 1 h. The protein bands were determined from the gel image system.

Quantitative polymerase chain reaction analysis on 3T3-L1 cells

The differentiation of preadipocytes to adipocytes involves the complex process of production and induction of different transcription factors (mRNA) which ultimately play a role in the expression of proteins for the lipid synthesizing and accumulation.[14] Gene expression of peroxisome proliferator-activated receptor gamma (PPARγ) is an important initiating factor for adipogenesis.[15] However, CCAAT enhancer binding protein alpha (C/EBPα) is also another important factor which activates PPARγ and play an important role in adipogenesis.[16] In this study, the levels of expression of transcription factors PPARγ, and C/EBPα on the differentiating 3T3-L1 cells with or without the treatment of EA and BuOH fraction was studied. The highest safe concentration of both fractions was used in the quantitative polymerase chain reaction (qPCR) experiment. The primers used in the experiments are shown in Table 1.

In vivo biological studies (anti-obesity study)

HF study on mice was done to evaluate the anti-obesity effect of *A. helferianai*. BuOH fraction of *A. helferiana* in two different doses (150 mg/kg/day and 300 mg/kg/day) was taken for the anti-obesity effect. Four-week-old male C57BL/6j mice, with average body weight of 20 g were purchased from Central Lab Animal Inc., (Seoul, Korea). Mice were maintained in accordance with the guidelines for the care and use of laboratory animals by Wonkwang University. The mice were housed under a 12 h light/12 h dark cycle in a temperature of 25°C ± 2°C and humidity of 55% ± 5%. They were allowed food and water *ad libitum* during the 10 weeks experiment period. After adaptation to the lighting conditions for a week, the mice were randomly divided into five dietary groups ($n = 7$) as follows:

1. Normal diet (ND) or normal group
2. HF or control group
3. BuOH fraction 150 (BF 150) group → HF supplemented with BuOH fraction 150 mg/kg
4. BuOH fraction 300 (BF 300) group → HF supplemented with BuOH fraction 300 mg/kg
5. *Garcinia cambogia* (GC 200) group → positive control (HF supplemented with *Garcinia cambogia* 200 mg/kg).

Samples (suspension in PBS) were administered orally. Normal and control group were fed with PBS per-oral. Body weights and food intake were recorded weekly. Blood glucose was recorded in every 3 weeks on lateral tail vein blood samples using an ACCU-Check glucose meter (Roche Diagnostics, Mannheim, Germany). Food efficiency ratio (FER) was calculated as follow:

FER = gained body weight (g) ×100/food intake (g) for 10 weeks %

Serum cholesterol and triglyceride

The concentration of total cholesterol (TC), triglycerides (TG), in serum were determined by using a commercial kit (AM 157S-K, Asan Pharm Co., Ltd, Whasung, Korea) in accordance with the manufacturer's instruction. Plasma aspartate aminotransferase (AST) and Alanine aminotransferase (ALT), levels in plasma were measured by using a commercially available kit (AM101-K; Asan Pharm., Korea). Renal function test was measured using diagnostic kits of blood urea nitrogen (BUN, AM 165-K, Asan Pharm Co., Ltd, Whasung, Korea) and creatinine (AM 119-K, Asan Pharm Co., Ltd, Whasung, Korea). All experiments were assayed by the manufacturer's protocol and reaction solutions were analyzed using UV-vis spectrophotometer.

Histology

After the sacrifice, mice liver and white adipose tissues were immediately fixed in 10% buffered formaldehyde. They were embedded in paraffin after removal of water using alcohol dehydration. Serial of sections of the tissue (5 μm) were cut using a microtome, mounted on glass microscope slides and stained with hematoxylin and then stained with eosin. Then, they were examined using light microscope and images were captured on canon power shot A640 camera.

Statistical analysis

Statistical analysis was carried out using SPSS statistics 19 software (IBM Co., Armonk, NY, USA) measured using one-way analysis of variance (ANOVA) using Duncan's multiple range test, Dunnett's multiple comparisons test, and Student's *t*-test. Values of *P* were considered as statistical significance at the level of <0.05%.

RESULTS AND DISCUSSION

Extraction and fractionation

The dried rhizome of *A. helferiana* was extracted with methanol using heat and then fractionated with different solvents to give fractions as: Hexane, DCM, EA, BuOH and water [Figure 1]. The rhizome once extracted by methanol was again extracted with water to give water extract. The main purpose for re-extraction with water was to get highly polar components still left in the rhizome which were hard to get extracted by using methanol. Interestingly, we got considerable amount of water extract. Since we got the highest amount of water extract and water fraction, this indicates that the rhizome of *A. helferiana* would

Table 1: The primer sequence used for real-time polymerase chain reaction

Primers	Sequences	
	Forward	Reverse
mPPARγ	5'-GTG AAG CCC ATC GAG GAC A-3'	5'-TGG AGC ACC TTG GCG AAC A-3'
mC/EBPα	5'-GCG GGA ACG CAA CAA CAT C-3'	5'-GTC ACT GGT CAA CTC CAG CAC-3'
mβ-actin	5'-GTG ACG TTG ACA TCC GTA AAG A-3'	5'-GCC GGA CTC ATC GTA CTC C-3'

contain a large quantity of water-soluble constituents such as saponis, glycosides, and tannins.[6] However, the biological activities of aqueous and nonaqueous extract may vary according to the presence of active constituents.[17,18]

Total flavonoid and polyphenol content test

The results of total flavonoid and polyphenol content are given in Table 2. The total flavonoid content was calculated from the calibration plot and expressed as milligrams of QE per gram of extract or fraction. Similarly, the total phenolics content was expressed as GAE in milligrams per gram of extract. EA fraction of rhizome showed the highest level of flavonoids and polyphenols. After that, the water extract showed the highest level of flavonoids and polyphenols followed by BuOH fraction. The data also reveal that the crude extracts of rhizome had higher flavonoid and polyphenol content than that of aerial parts. This supports the higher medicinal value of rhizome of *A. helferiana* than the aerial parts.

Table 2: Total flavonoids and polyphenol content expressed as quercetin equivalent and gallic acid equivalents, respectively

Sample	QE	GAE
Rhizome		
Crude extract	23.93±0.21	16.01±1.98
Hexane fraction	20.95±0.34	22.69±2.37
DCM fraction	19.12±0.91	31.31±3.55
EA fraction	106.9±0.83	92.81±3.33
BuOH fraction	55.09±0.26	54.58±1.99
Water fraction	7.31±0.05	13.76±2.03
Water extract	71.85±0.55	61.16±2.67
Leaf and petiole		
Crude extract	10.12±1.23	8.70±1.08

Values are expressed as mean±SD (n=3). GAE: Gallic acid equivalents; QE: Quercetin equivalent; DCM: Dichloromethane; EA: Ethyl acetate; BuOH: Butanol; SD: Standard deviation

Antioxidant and anti-inflammatory activity

Flavonoids and polyphenols are the good source of the antioxidant and anti-inflammatory component in plants which help to fight against various diseases resulting from the oxidative stress.[19] *A. helferiana* being a good source of flavonoids and polyphenols was assessed for the antioxidant and anti-inflammatory activity. Antioxidant activity of extract and fractions were evaluated using DPPH assay. The EA and BuOH fraction were found to possess strong antioxidant activity as evident from their very small IC_{50} values [Figure 2] compared to others. However, the water extract also showed good antioxidant activity. The higher antioxidant activity of EA, BuOH, and water extract correlated with their higher polyphenol and flavonoid content. Like in other studies of fern, the rhizome extract of *A. helferiana* were stronger radical scavengers (data not shown) than the aerial part extracts.[20]

All the extract and fractions were evaluated for NO scavenging activity and only the EA fraction of rhizome of *A. helferiana* was found to be highly potent (data not shown). To further establish the anti-inflammatory activity, we continued the experiment in cellular levels for the EA fraction. The anti-inflammatory activity of EA fraction was assayed against RAW 264.7 macrophage cells for the inhibition of nitric oxide radical production after the induction by LPS. The viability assay of EA fraction on RAW 264.7 macrophage cells was done [Figure 2b], and the NO scavenging activity was evaluated [Figure 2c]. The EA fraction significantly inhibited

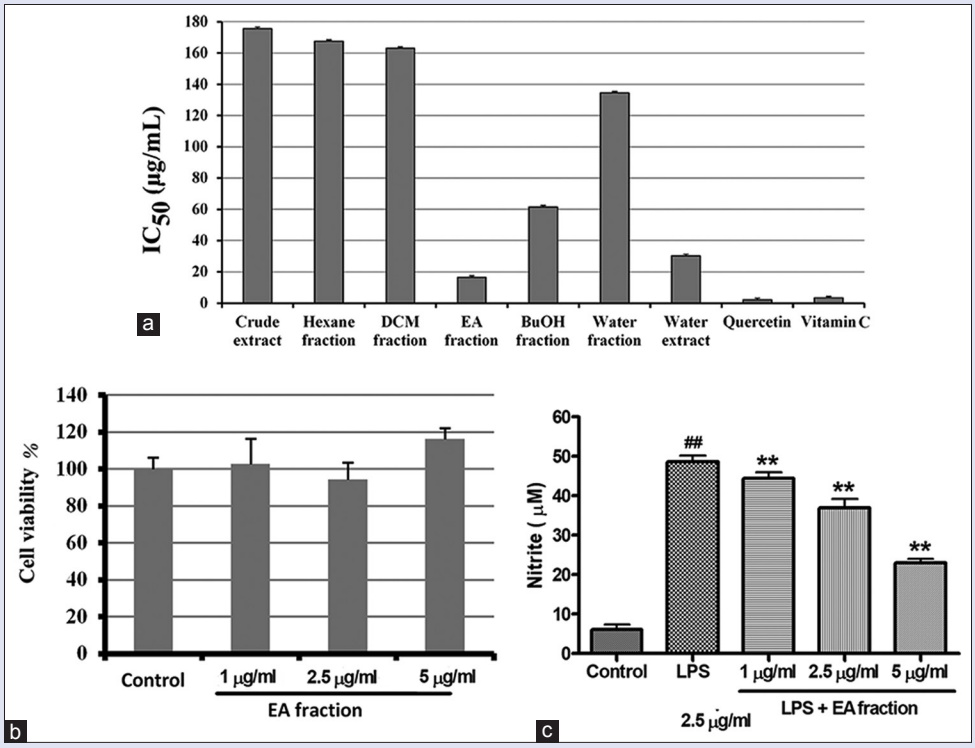


Figure 2: Antioxidant activity of extract/different fraction of *Angiopteris helferiana* rhizome and standard were estimated by 1, 1-diphenyl-2-picryl-hydrazyl free radical scavenging test and the results were expressed as IC_{50} values (a). Values are expressed as mean ± standard deviation (n = 3). Nitric oxide radical scavenging assay in lipopolysaccharide-stimulated RAW 264.7 cells was determined after the viability assay (b) of ethyl acetate fraction on RAW 264.7 cells. The production of nitrate ions after treating RAW 264.7 cells with lipopolysaccharide alone or with different concentration of ethyl acetate fraction was evaluated (c). The data shown are represented as the mean ± standard error of the mean of three separate experiments. Statistical significance was calculated using one-way ANOVA followed by Dunnett's multiple comparisons test. $^{\#\#}P < 0.01$ versus control; $^{**}P < 0.01$ versus LPS

the nitrite production in LPS-stimulated RAW 264.7 cells. The inhibition was found to increase on concentration-dependent manner.

In vitro α-glucosidase inhibition assay

Some studies have revealed that ferns also have good α-Glucosidase inhibition activity.[21] In this study, the α-Glucosidase inhibition assay of the extract and fractions of *A. helferiana* was evaluate to know its potential for being a good source of antidiabetic medicine. Several α-glucosidase inhibitors, such as acarbose and voglibose obtained from natural sources, can effectively control blood glucose levels after food intake and have been used clinically in the treatment of diabetes mellitus.[22] All the extract and fractions showed a good effect against the α-glucosidase enzymes compared to the positive control, i.e., acarbose [Table 3]. However, EA fraction followed by water extract and BuOH fraction showed significantly higher inhibition of α-glucosidase (EC_{50} 10.64 ± 0.35, 31.69 ± 0.39, 10.64, and 69.84 ± 0.60, respectively). This suggests that plant *A. helferiana* would be a potential source of oral anti-diabetic medicine. As the aqueous extract has shown good α-glucosidase inhibition activity, we can easily obtain the α-glucosidase inhibitors from *A. helferiana* by simple water extraction procedure.

Table 3: IC_{50} values of extract/different fraction of *Angiopteris helferiana* rhizome and standards obtained from α - glucosidase inhibition assay

Rhizome	IC_{50} (μg/mL)
Crude extract	73.02±0.52
Hexane fraction	71.95±0.89
DCM fraction	90.32±3.87
EA fraction	10.64±0.35
BuOH fraction	31.69±0.39
Water fraction	69.84±0.60
Water extract	18.81±1.33
Acarbose	2505.01±2.09

Values are expressed as mean±SD (n=3). SD: Standard deviation; DCM: Dichloromethane; EA: Ethyl acetate; BuOH: Butanol

Inhibition of lipid in differentiating 3T3-L1 cells

All the extract and fractions of rhizome and leaf of *A. helferiana* were studied for antiadipogenic activity on 3T3-L1 cells. After the viability assay, the differentiating cells were treated with or without different concentration of crude extract and fractions of rhizome and leaf of *A. helferiana*. Among the different extract and fractions, only the EA and BuOH fraction of rhizome of *A. helferiana* showed promising activity and the results are shown in Figure 3. The lipid accumulation in 3T3-L1 adipocytes treated with or without different concentration of EA [Figure 3a] and BuOH [Figure 3b] was visualized using ORO staining. The quantitative data of lipid production after the ORO staining were also evaluated for the EA [Figure 3c] and BuOH [Figure 3d] fraction. Both EA and BuOH fraction of the rhizome showed increase in inhibition of lipid production on concentration-dependent manner.

Inhibition of adipogenic markers in differentiating 3T3-L1 cells

Since EA and BuOH fraction of rhizome showed good results of lipid inhibition in the ORO study, further analysis was performed to see the effects on the expression of major adipogenic factors. The differentiation

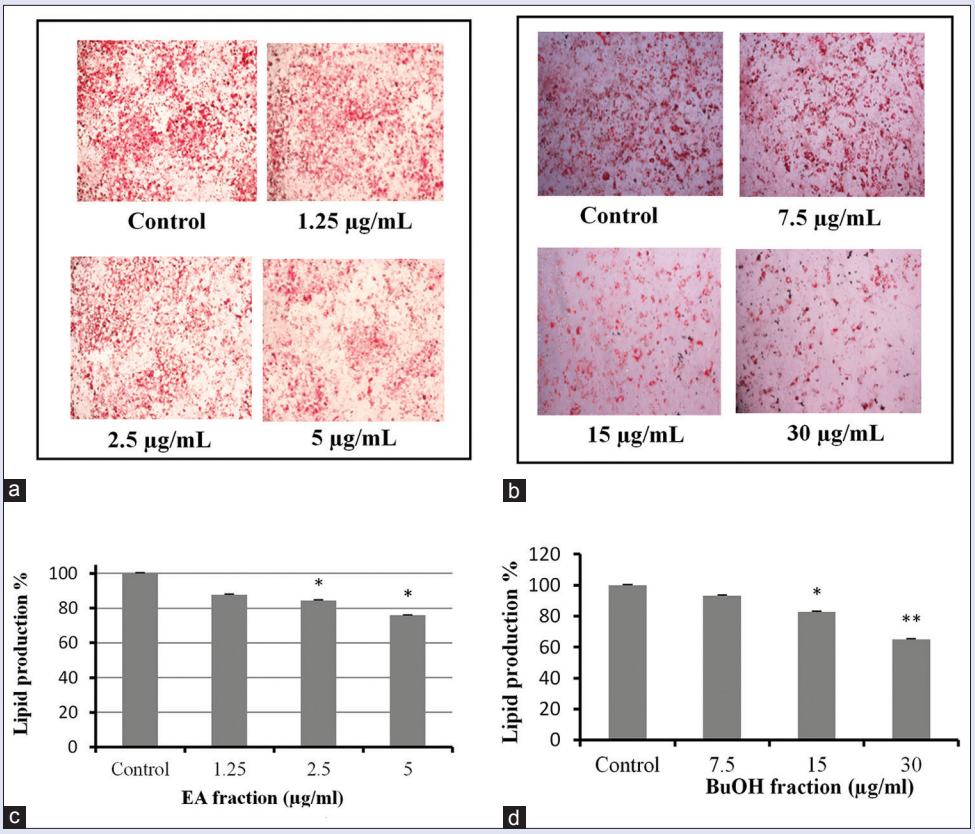


Figure 3: Oil red O staining of 3T3-L1 cells after differentiation with or without the presence of ethyl acetate fraction (a) and butanol fraction (b). Quantitative analysis of the reduction of lipid production by ethyl acetate fraction (c) and butanol fraction (d). Mean ± standard error of the mean from three independent experiments, Student's *t*-test (*P < 0.05 and **P < 0.01)

of preadipocytes to adipocytes involves the complex process of production and induction of different transcription factors (mRNA) which ultimately play a role in the expression of proteins for the lipid synthesis and accumulation.[14] Gene expression of PPARγ is an important factor for the initiation of differentiation in 3T3-L1 cells.[15,23] However, C/EBPα is also another important factor which activates PPARγ and enhances lipid production in adipogenesis.[16,24] We studied the effect of EA and BuOH fraction of rhizome on these regulators during the adipogenesis of 3T3-L1. The western blotting results [Figure 4a] showed the EA and BuOH fractions, significantly inhibiting the protein level of the major adipogenic factors PPARγ and C/EBPα in the differentiated 3T3-L1 cells compared to the control (nontreated cells). After the promising results of inhibition of expression of adipogenic factors by the EA and BuOH fractions of the rhizome of *A. helferiana* from western blotting, we tried to investigate the effect of EA and BuOH fractions on the adipogenic transcription factors PPARγ, and C/EBPα in differentiating 3T3-L1 cells by qPCR analysis. The highest safe concentration of both fractions was used in the qPCR experiment. There was significant decrease in expression of the transcriptions factors by both fractions [Figure 4b and 4c]. This clearly indicated the significant antiadipogenic activity of both fractions.

Animal study

The effect of BuOH fraction on the body weight gain, food intake, and FER were examined and given in Table 4. The final body weight of the HF group (35.32 ± 1.312 g) increased by 66% whereas for ND group (24.95 ± 0.46 g), it was only 20%. The significant difference of body weight between HF and ND group indicated an obese condition in HF group. Mice fed with BuOH 150 did not show sufficient decrease in body weight. However, BuOH 300 and GC 200 (positive control) significantly reduced the body weight compared to HF group. There was only around 50% body weight gain in BuOH 300 and GC 200 group. This indicates that supplementation of BuOH fraction inhibited the body weight gain resulting from the HF.

The food intake pattern was similar for all groups apart from ND group which was a little bit high. HF group showed higher FER indicating that most of the food taken contributed in weight gain. The other groups showed lower FER than that of HF group. The lower FER suggested effective lipid metabolism which helps to maintain the body weight despite high calories intake. From the body weight and FER data [Table 4], the low FER values for BuOH 300 suggested its effective role in lipid metabolism.

Effects on organ weight and serum toxicity markers

The results of organ weight of different groups of mice are also given in Table 4. HF and BuOH fed group showed decrease in weight of liver compared to ND group. This created a doubt if the decrease in liver weight was due to toxicity in the liver. The liver toxicity markers (AST and ALT) were analyzed to identify liver damage.[25] Plasma AST and ALT levels of mice of the HF were found to be significantly higher than that of ND [Table 5]. This indicated some level of liver toxicity in HF mice that may have resulted from the development of obesity. Plasma ALT and AST levels of BuOH treated groups (20.50 ± 3.44 U/L and 116.00 ± 10.77 U/L)

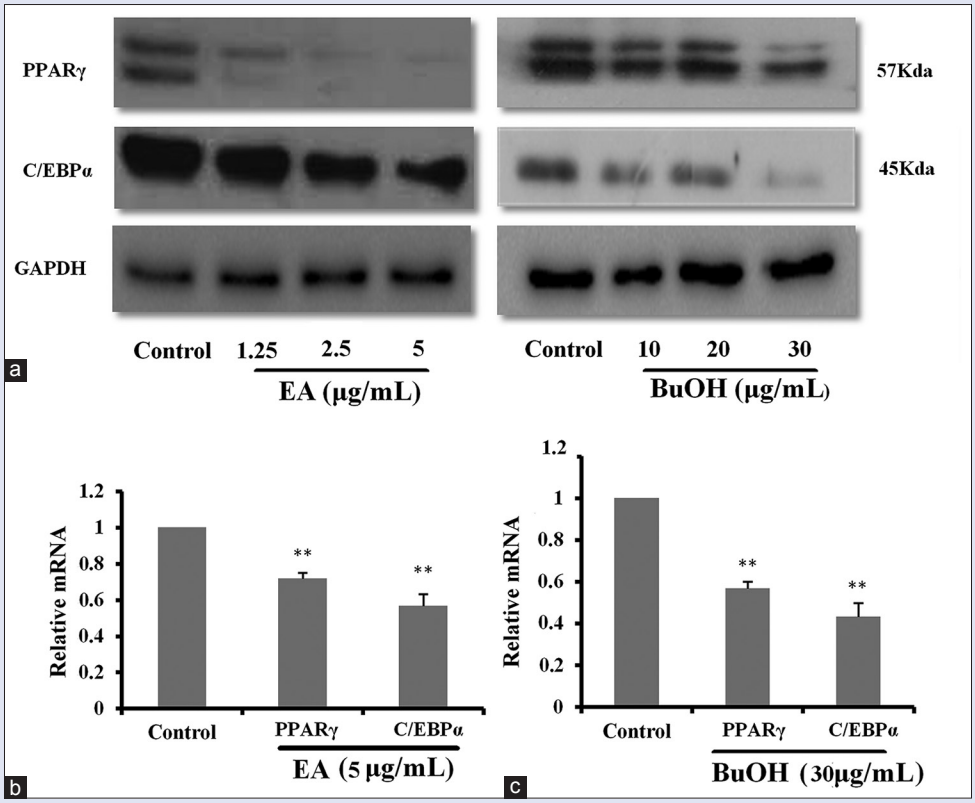


Figure 4: Western blotting analysis was performed to see the effects of different concentrations of ethyl acetate fraction and butanol fraction on PPARγ and C/EBPα proteins expression in 3T3-L1 cells (a). 3T3-L1 cells were differentiated with or without the presence of Ethyl acetate fraction (5 µg/mL) and butanol fraction (30 µg/mL) of the rhizome of *Angiopteris helferiana* and mRNA expression of PPARγ and C/EBPα was measured by quantitative real-time polymerase chain reaction EA fraction (b) and Butanol fraction (c) significantly inhibited the expression of mRNA of PPARγ, and C/EBPα. Data shown represent the mean ± standard error of the mean from three independent experiments. Statistical significance was determined relative to a control by the Student's *t*-test (*$P < 0.05$; **$P < 0.005$)

Table 4: The effect of samples on the body weight, weight gain, food intake food efficiency, and organ weight

Arameters	ND	HF	GC 200	BuOH 150	BuOH 300
Initial body weight (g)	20.83±0.65[a]	21.37±1.28[a]	21.088±0.78[a]	20.331±1.09[a]	20.63±1.12[a]
Final body weight (g)	24.95±0.46[a]	35.32±1.31[c]	32.785±1.94[b,c]	33.37±2.21[b,c]	31.61±2.11[b]
Body weight gain (g)	4.128±0.49[a]	14.11±1.19[c]	11.69±2.21[b,c]	13.41±1.84[b,c]	10.98±2.26[b]
Body weight gain (%)	20	66	52	65	50
Food intake (g/day)	0.363	0.21	0.28	0.252	0.281
FER (%)	16.23	77.21	58.58	61.48	46.46
Liver (mg)	959.23±33.47[a]	900.21±9.44[a,b]	966.41±57.84[a]	907.77±33.46[a,b]	875.63±35.06[b]
Spleen (mg)	62.21±6.04[a]	71.58±3.14[b]	69.53±4.72[b]	58.1±4.15[a]	61.34±6.86[a]
Kidney (mg)	309.46±8.35[a,b]	335.23±13.94[b]	330.76±34.91[b]	292.35±17.11[a]	300.57±10.72[a]

FER (%): Food efficiency ratio; ND: Normal diet; HF: High-fat diet; GC: *Garcinia cambogia*; BuOH: Butanol

Table 5: Alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, and creatinine levels

Parameters	ND	HF	GC 200	BuOH 150	BuOH 300
ALT-L (U/L)	27.60±4.49[a]	39.00±2.44[b]	37.16±9.02[b]	27.00±6.75[a]	20.50±3.44[a]
AST-L (U/L)	145.50±12.59[a,b]	185.50±14.52[c]	152.75±29.86[a,b]	139.00±26.49[a,b]	116.00±10.77[a]
CRE (U/L)	0.208±0.02[a]	0.208±0.02[a]	0.206±0.02[a]	0.173±0.03[b]	0.155±0.01[b]
BUN (U/L)	38.33±3.44[a]	36.71±2.36[a]	36.3±1.78[a]	37.37±2.57[a]	37.75±6.11[a]

All values are express as the mean±SD. The effects of samples are compared by one-way ANOVA using Duncan's multiple range test. Values with different letters in a row are significantly different ($P<0.05$) based on one-way ANOVA *post hoc* Ducan multiple range tests. ANOVA: Analysis of variance; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BUN: Blood urea nitrogen; ND: Normal diet; HF: High-fat diet; GC: *Garcinia cambogia*; BuOH: Butanol; SD: Standard deviation; CRE: Creatinine

were found to be significantly lower ($P < 0.05$) compared to that of the HF group (39.00 ± 2.44 U/L; $P < 0.05$ and 185.50 ± 14.52 U/L). These data assured that the BuOH fraction fed mice had no such kind of liver toxicity as in HF-fed group. The weight of spleen in HF group is little more than normal [Table 4]. The reason for abnormal enlargement of spleen might be due to obesity-induced inflammation in HF group which was in consistent with the results of a study in rats.[26] In BuOH fractions, spleen weight was found normal. No toxic symptoms or mortality were observed in any animals after the administration of the fraction at varied doses of 150 and 300 mg/kg throughout the experiment period of 10 weeks. Hence, the doses of BuOH fraction administered to the mice were safe for the liver and spleen.

In the case of BUN and creatinine level, there was no significant difference among the groups indicating normal renal functions in all groups. Hence, the BuOH fraction supplementation was also safe for the kidney.

Effects on blood glucose, serum lipid, and fat deposition (in the liver and adipose tissue)

During the 10 weeks period of the experiment, the blood glucose was measured in every 3 weeks interval [Figure 5a]. Initially, all groups had similar blood glucose level. The HF showed large increase in blood glucose level at the end of the study. The GC and BuOH samples were able to reduce the blood glucose level. The BuOH 300 showed dramatic reduction of glucose level. The last measurement was done at fasting condition, and hence, the values are a little bit lower than the expected. This indicated that the BuOH fraction can be a potential source of antidiabetic remedy.

The results in Figure 5b and c show the significant increase in serum lipid (TG and TC) in HF group compared to the normal group indicating an obese state in the HF group. The supplementation of BuOH fraction of *A. helferiana* reduced the lipid metabolism as evidenced by the decreased level of TG and TC in BuOH 150 and 300 groups.

The obese condition of HF group is evidenced from the data of white adipose tissue [Figure 5d] which shows large difference between the HF and ND group. The supplementation of GC and BuOH fraction reduced the fat accumulation in the adipose tissue resulting in lower white adipose tissue mass compared to the HF group. Among the three samples, BuOH 300 showed the highest reduction (around 50%) of fat compared to HF group. The size of adipocyte cells in adipose tissue of different groups of mice can be seen in the histology (hematoxylin and eosin staining) of white adipose tissue in Figure 5e. HF group showed significant increase in adipocyte area/size compared to ND group. The GC 200 (positive control) and BuOH 150 group did not show significant decreases in adipocyte area size compared to HF group. However, the BuOH 300 group showed significant decrease in adipocyte area size compared to HF group. We presume in consideration of these results that the decrease in body fat mass by BuOH treatment is partly due to the decrease in adipocyte size.

Hematoxylin and eosin staining of liver sections of all groups of mice are shown in Figure 5f. The histology of ND group showed normal liver structure with some macrovesicular fat accumulation. HF group mice livers displayed minor to major microvesicular steatosis (lipid accumulation). HF group also showed the presence of a large number of circular lipid droplets. These lipid droplets are strikingly reduced in both size and number in the liver of BuOH 300 group suggesting that BuOH 300 treatment effectively inhibited the lipid accumulation in the liver. GC 200 and BuOH 150 also reduced the fat accumulation in the liver, but the results are not considerable.

DISCUSSION

A. helferiana can be considered as a medicinal fern according to its known traditional medicinal values in the rural communities. We have been able to explore many bioactivites for the first time regarding this plant. The plant was found to have the potent antioxidative capacity and α-glucosidase activity. We know that α-glucosidase inhibitors reversibly inhibit a number of α-glucosidase enzymes (e.g., maltase), consequently delaying the absorption of sugars from the gut.[27] Hence, α-glucosidase inhibitors are used in the treatment of diabetes mellitus oral hypoglycemic agents.[28] Interesting thing is that the antioxidant and α-glucosidase activity of the water extract (obtained after reextraction) was extremely higher than that of the water fraction (obtained from the fractionation of methanol extract). It ranked third after EA and BuOH fraction. Hence, it assured that simple boiling of *A. helferiana*

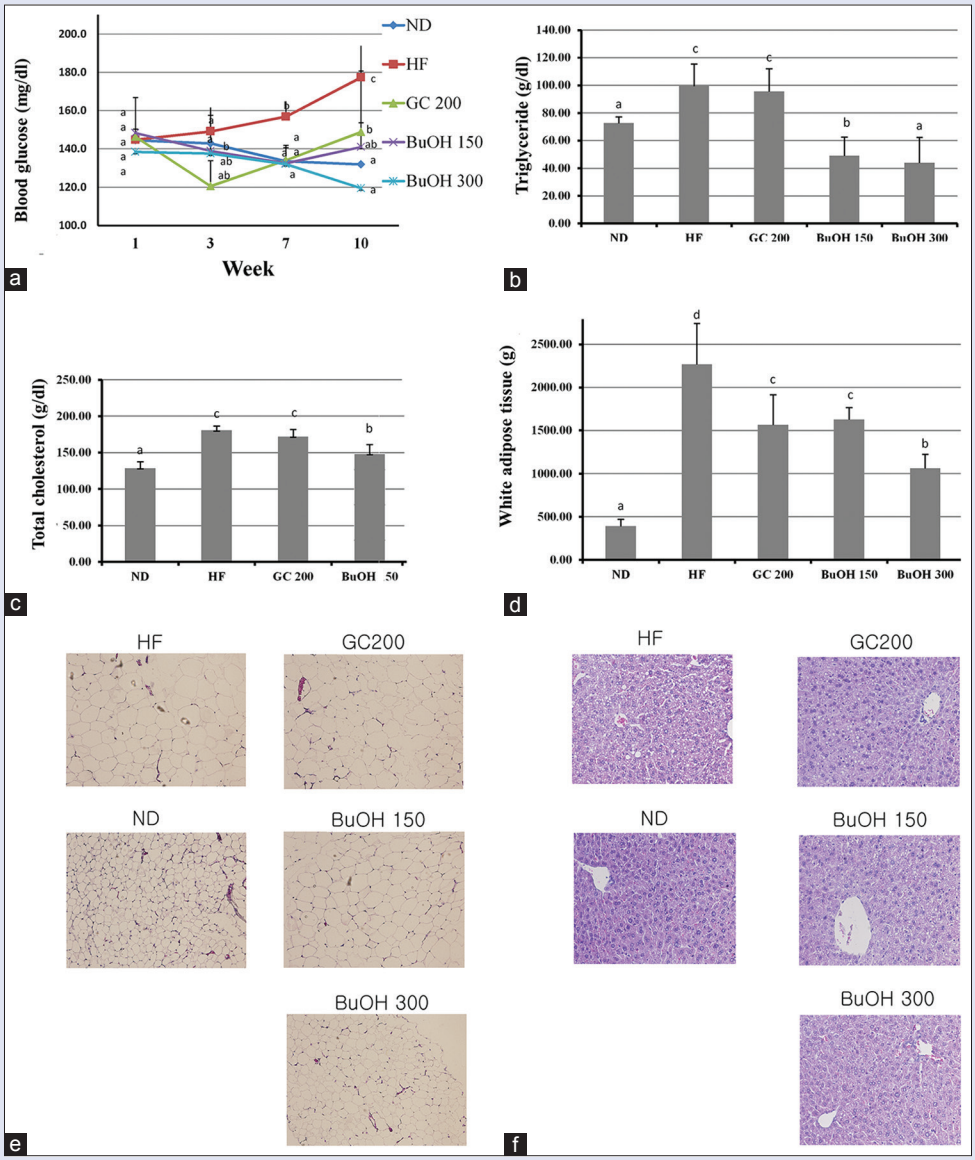


Figure 5: Blood glucose level of all groups was measured at different time during the experiment (a). The serum of all groups was analyzed for the level Triglyceride (b) and Total cholesterol (c). The weight of adipose tissue (epididymal fat) of all groups was evaluated to observe the lipid accumulation in adipose tissue (d).Values are expressed as means ± standard error of the mean with same letters indicate no significant differences at $P < 0.05$ according to one-way ANOVA *post hoc* Ducan Multiple Range tests. Representative histology photographs of epididymal white adipose tissue (e) and hepatic tissue (f) of mice were captured after staining. high-fat diet-high fat diet group; normal diet-normal diet group; *Garcinia cambogia* 200-high fat diet + Garcinia cambogia extract 200 mg/kg/day; butanol 150-high fat diet + butanol fraction 150 mg/kg/day; butanol 300-high fat diet + butanol fraction 300 mg/kg/day. Sections were examined by light microscopy at a magnification of × 200 magnification

rhizome in water and preparation of water extract would be an efficient anti-oxidative and anti-diabetic supplement.

The *in vivo* animal experiment also showed the glucose lowering activity in blood in HF fed mice supplemented with the BuOH fraction of *A. helferiana*. Hence, this further established its potent anti-diabetic effect.

The literature survey indicated that very few ferns have been reported with antiobesity activity. Hence to explore fern with antiobesity activity is a great achievement in itself. We studied the antiobesity activity in both cell and animal model and found significant inhibition of lipid production. The EA and BuOH fraction significantly inhibited the markers PPARγ and C/EBPα, which are very essential for lipid synthesis in 3T3-L1 cells. Actually, PPARγ and C/EBPα are the important factor for the initiation of differentiation in 3T3-L1 cells.[14,15,23] There was good inhibition of lipid accumulation in the liver and adipose tissue after the supplementation of BuOH fraction in the HF fed mice. The lipid in blood serum was also decreased by the fraction supplementation.

CONCLUSION

Hence, there is no doubt that, *A. helferiana* would be promising bioresources of antioxidant, anti-inflammatory, antiadipogenic, and anti-diabetic components. Our findings have added valuable information to the currently limited knowledge *A. helferiana*. It has great scope for the discovery of bioactive compounds that can be used for the preparation of nutraceuticals, cosmetics,

and pharmaceutical products which could be used for different remedies.

Acknowledgement

We give our sincere thanks to the Bio andamp; Medical Technology Development Program of the National Research Foundation (NRF) and the Korean government for funding our study.

Financial support and sponsorship

This research work is funded by Wonkwnag University, Iksan, Jeonbuk, S. Korea and Korean government (MSIP and amp; MOHW) (NRF432 2015M3A9A5031098).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Ho R, Teai T, Bianchini JP, Lafont R, Raharivelomanana P. Ferns: From traditional uses to pharmaceutical development, chemical identification of active principles. In: Fernández H, Kumar A, editors. Working with Ferns: Issues and applications. New York: Springer; 2011. p. 321-46.
2. Ding ZT, Fang YS, Tai ZG, Yang MH, Xu YQ, Li F, *et al.* Phenolic content and radical scavenging capacity of 31 species of ferns. Fitoterapia 2008;79:581-3.
3. Soare LC, Ferdes M, Stefanov S, Denkova Z, Nicolova R, Denev P, *et al.* Antioxidant activity, polyphenols content and antimicrobial activity of several native pteridophytes of Romania. Notulae Bot Hortic Agrobotanici Clujul Napoca 2012;40:53.
4. Fraser-Jenkins CR, Kandel DR Pariyar S. Ferns and Fern-Allies of Nepal. Kathmandu: National Herbarium and Plant Laboratories, Department of Plant Resources, Ministry of Forest and Soil Conservation; 2015.
5. Wu ZY, Raven PH, Hond DY, editors. Flora of China. Beijing and St. Louis: Science Press and Missouri Botanical Garden Press; 2013.
6. Huzaifa U, Labaran I, Bello A, Olatunde A. Phytochemical screening of aqueous extract of garlic (*Allium sativum*) bulbs. Rep Opin 2014;6:1-4.
7. Balendra PS, Ravi U. Medicinal pteridophytes of Madhya Pradesh. J Pharmacogn Phytochem 2014;3:173-6.
8. Shaikh SD, Masal VP, Shaikh AS. Ethnomedicinal uses of some pteridophytes from Konkan region of Maharashtra, India. Int J Pharm Chem Sci 2014;3:63-5.
9. Chen Y, Tao Y, Lian X, Wang L, Zhao Y, Jiang J, *et al.* Chemical constituents of *Angiopteris esculenta* including two new natural lactones. Food Chem 2010;122:1173-5.
10. Arvouet-Grand A, Vennat B, Pourrat A, Legret P. Standardization of propolis extract and identification of principal constituents. J Pharm Belg 1994;49:462-8.
11. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. Am J Enol Vitic 1965;16:144-58.
12. Hazra B, Sarkar R, Biswas S, Mandal N. Comparative study of the antioxidant and reactive oxygen species scavenging properties in the extracts of the fruits of *Terminalia chebula*, *Terminalia belerica* and *Emblica officinalis*. BMC Complement Altern Med 2010;10:20.
13. Mccue P, Kwon YI, Shetty K. Anti-amylase, anti-glucosidase and anti-angiotensin i-converting enzyme potential of selected foods. J Food Biochem 2005;29:278-94.
14. Farmer SR. Transcriptional control of adipocyte formation. Cell Metab 2006;4:263-73.
15. Rosen ED, Spiegelman BM. Adipocytes as regulators of energy balance and glucose homeostasis. Nature 2006;444:847-53.
16. Rosen ED, Hsu CH, Wang X, Sakai S, Freeman MW, Gonzalez FJ, *et al.* C/EBPalpha induces adipogenesis through PPARgamma: A unified pathway. Genes Dev 2002;16:22-6.
17. Bae JY, Lee YS, Han SY, Jeong EJ, Lee MK, Kong JY, *et al.* A comparison between water and ethanol extracts of *Rumex acetosa* for protective effects on gastric ulcers in mice. Biomol Ther (Seoul) 2012;20:425-30.
18. Ju JB, Kim JS, Choi CW, Lee HK, Oh TK, Kim SC, *et al.* Comparison between ethanolic and aqueous extracts from Chinese Juniper berries for hypoglycaemic and hypolipidemic effects in alloxan-induced diabetic rats. J Ethnopharmacol 2008;115:110-5.
19. Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, *et al.* Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem 1999;47:3954-62.
20. Valizadeh H, Sonboli A, Kordi FM, Dehghan H, Bahadori MB. Cytotoxicity, antioxidant activity and phenolic content of eight fern species from North of Iran. Pharm Sci 2015;21:18.
21. Chai TT, Yeoh LY, Ismail NI, Ong HC, Manan FA, Wong FC. Evaluation of glucosidase inhibitory and cytotoxic potential of five selected edible and medicinal ferns. Trop J Pharm Res. 2015;14:449-54.
22. Playford RJ, Pither C, Gao R, Middleton SJ. Use of the alpha-glucosidase inhibitor acarbose in patients with Middleton syndrome: Normal gastric anatomy but with accelerated gastric emptying causing postprandial reactive hypoglycemia and diarrhea. Can J Gastroenterol Hepatol 2013;27:403-4.
23. Rosen ED, Walkey CJ, Puigserver P, Spiegelman BM. Transcriptional regulation of adipogenesis. Genes Dev 2000;14:1293-307.
24. Wu Z, Rosen ED, Brun R, Hauser S, Adelmant G, Troy AE, *et al.* Cross-regulation of C/EBP alpha and PPAR gamma controls the transcriptional pathway of adipogenesis and insulin sensitivity. Mol Cell 1999;3:151-8.
25. M'Kada H, Munteanu M, Perazzo H, Ngo Y, Ramanujam N, Imbert-Bismut F, *et al.* What are the best reference values for a normal serum alanine transaminase activity (ALT)? Impact on the presumed prevalence of drug induced liver injury (DILI). Regul Toxicol Pharmacol 2011;60:290-5.
26. Health Promotion Board. National Nutrition Survey Health Promotion Board. Singapore: Health Promotion Board; 2010.
27. Campbell LK, White JR, Campbell RK. Acarbose: Its role in the treatment of diabetes mellitus. Ann Pharmacother 1996;30:1255-62.
28. Asano N. Glycosidase inhibitors: Update and perspectives on practical use. Glycobiology 2003;13:93R-104R.