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ANTIOBESITY POTENTIAL OF CLERODENDRUM PHLOMIDIS LINN AND GARCINIA CAMBOGIA LINN –A COMPARATIVE ANIMAL MODEL STUDY

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ABSTRACT

Obesity is one of the major health concerns in the 21st century and it is a major and leading cause of coronary heart diseases, stroke, type 2 diabetes, atherosclerosis and certain cancers. Available literature survey reveals that plant based drugs are much sought after therapeutic agents to prevent and cure many diseases including obesity. **Objective**: To evaluate the antiobesity potential of the *Clerodendrum phlomidis* leaf extract and its activity was compared with a known herbal antiobesity drug, *Garcinia cambogia* fruit extract in High Fat Diet (HFD) induced obese animal model. Orlistat was used as standard drug. **Methods**: Oral administration of *Clerodendrum phlomidis* leaf extract and *Garcinia cambogia* fruit extract (400mg/kg-bw) on HFD induced obese rats were taken for assessing (1) Behavioral parameters: ambulation, rearing and grooming; (2) Physiological parameters: weight of the animals, organs, fat pads and body temperature; (3) Biochemical parameters: blood glucose, total protein, Total lipids, free fatty acids and uric acid; (4) Liver function markers: AST, ALT and ALP and (5) Lipid profiles**:** serum cholesterol, serum triglycerides, LDLc, VLDLc, and HDLc. **Results**: HFD induced obesity to rats, and the obese rats, showed significant increase in the levels of physiological and biochemical parameters, liver function markers and lipid profiles and co-administration of *Clerodendrum phlomidis* leaf extract and *Garcinia cambogia* fruit extract significantly reduced the levels of all parameters and brought back normalcy. But the levels of total protein and HDLc were decreased in obese rats and the same were increased up to the levels of healthy rats after the administration of plant drugs. The animals treated with standard drug-Orlistat, also showed ameliorating effect in the obese

rats. **Conclusion**: The present study revealed that *Clerodendrum phlomidis* leaf extract is a effective antiobesity drug and its efficacy was comparable with a known antiobesity plant drug, *Garcinia cambogia* fruit extract and a standard drug, Orlistat. It is already recorded that (-) hydroxy citric acid is the active phytochemical available in *Garcinia cambogia* fruit extract, which is responsible for its antiobesity activity. In the present study ursolic acid was isolated and identified as pharmacologically active compound in *Clerodendrum phlomidis* leaf extract for its antiobesity activity.

KEY WORDS: *Clerodendrum phlomidis*, *Garcinia cambogia*, Orlistat, hydroxy citric acid, ursolic acid, antiobesity activity.

INTRODUCTION

Obesity and overweight are become the serious global problems in recent years. It is a major public health issue and increasing prevalence across all sex, age groups and ethnicity or race. The recent report of WHO (2012) estimated that worldwide 1.5 billion adults are overweight, among them over 200 million men and almost 300 million women are obese [1]. Obesity and its co-morbid disorders are the leading causes for premature mortality in developed countries. Obese persons are also vulnerable to low self-esteem and depression because of the psychological and social stigmata that are associated with obesity. On a global scale, obesity has reached epidemic proportions and is a major contributor to the global burden of chronic disease and disability[1]. Obesity is developed because of chronic disorders of cholesterol and carbohydrate metabolism and it is characterized by excess fat deposition in adipose tissues, heart, liver, skeletal muscles, and islets of pancreas [2]. Overweight and obesity are having increasing risk of numerous chronic diseases, including coronary heart disease, type 2 diabetes, stroke, dyslipidemia, cardiovascular diseases, metabolic syndrome, cholelithiasis, gall bladder cancer and some other type of cancers.

In India, obesity is the most prevalent in urban populations (male: 5.5%, female: 12.6%), followed by the urban slums (male: 1.9%, female: 7.2%). Obesity rates are the lowest in rural populations (male: 1.6%, female: 3.8%). Obesity has reached epidemic proportions in India with morbid obesity affecting 5% of the country's population. Socioeconomic growth has an impact on the rate of obesity. As populations become more westernized, concerns about obesity epidemic are on the rise [3]. Onset of obesity is a result of energy imbalance, which leads to a gradual but persistent weight gain over a considerable period. Physical activity, chronic alcoholism, overconsumption of food and sedentary lifestyle are important

contributing factors in the development of obesity. Sugars and fats are mainly providing energy (calorie). If calorie intake is in excess of calorie output, it will be stored mainly as body fat. If the stored body fat is not utilized over time, it will lead to overweight or obesity. Body mass index (BMI) is a simple index of weight-for-height that is commonly used to classify overweight and obesity in adults. It is defined as a person's weight in kilograms divided by the square of his height in meters (weight in kg /height in m^2). The WHO definition is: BMI greater than or equal to 25 is overweight and BMI greater than or equal to 30 is obesity. Presently, body mass index (BMI) and waist circumference (WC) are two criteria that have been employed for classifying obesity.

Herbal drugs have been used since ancient times as medicines for the treatment of wide range of diseases and the medicinal plants have played a key role in world health. In spite of the great advances observed in modern medicine in recent years, plants still make an important contribution to health care. Medicinal plants are distributed worldwide, but they are most abundant in tropical countries. It is estimated that about 25% of all modern medicines are directly or indirectly derived from higher plants. In some particular cases, such as antitumor and antimicrobial drugs, about 60% of the medicines currently available on the market and most of those in the late stages of clinical trials are derived from natural products, mainly from plants. Our earlier investigation on antidiabetic activity of *Clerodendrum phlomidis* leaf extract showed antihyperlipidemic activity [5]. So the present study was aimed to investigate the antiobesity potential of *Clerodendrum phlomidis* leaf extract and compare its activity with a known antiobesity herbal drug *Garcinia cambogia* fruit extract and also with a standard reference drug, Orlistat. *Clerodendrum phlomidis* Linn. (syn: *Clerodendrum multiflorum* (Burm.F) Kuntz.; *Volkameria multiflorum* Burm. F.; Botanical Family: Lamiaceae) is a perennial, erect, bushy, herbaceous shrub that grows in arid plains, low hills, drier parts and tropical deserts of India. The vernacular names are Sanskrit: Agnimantha, Hindi: Arni, Tamil: Thazhuthalai, Telugu: Tekkali, Malayalam: Tiruthalai. The *Clerodendrum phlomidis* leaf was used as remedy to treat against diabetes in southern parts of India especially by the tribes of Nilgiris, Tamil Nadu. The traditional uses of leaf juice are to treat in dyspepsia, fever, hypertension, high cholesterol and for stimulating intestine as peristalsis agent[4]. *Garcinia cambogia* Linn. (Botanical family: Gutiferae) is a small medium size tree with round crown and horizontal or drooping branches; leaves are dark green, shiny and fruits are ovoid , yellow or red when ripe with 6 or 8 grooves and 6 or 8 seeds. The tree is growing in the evergreen forests of Western Ghats, from Konkan to Travancore, and in the Shola forests

of Nilgiris. The fruit is called as Kokum fruit, Malabar tamarind, Kudampuli and Malaipuli and it is used in South India as a condiment in foods. *Garcinia cambogia* fruit is reported to suppress appetite, boost metabolism, prevent fat deposition and reduce abdominal fat. In addition, it is reported to be useful in the following health problems: diabetes, diarrhea, gastrointestinal disorders, heart disease, high cholesterol and ulcers [4].

MATERIALS AND METHODS

Collection and Authentication of Plant Material

The leaves of *Clerodendrum phlomidis* Linn. were collected from the Sathuragiri Hills Area, Virudhunagar district of Tamil Nadu. Dried fruits of *Garcinia cambogia* Linn. were purchased from an authentic source from Bangalore. Both the herbal raw materials were identified and authenticated by the eminent professors in Botany Department and the voucher specimen (No. 062 and 064, respectively) were deposited in the department.

Preparation of Plant Extracts

1000 g of *Clerodendrum phlomidis* dried leaf was taken and extracted with 80% ethanol. To one part of the plant material six parts of 80% ethanol was added, extracted in round bottom flask fitted with condenser for 3 h at reflux temperature. The solvent extract was filtered and the filtrate was evaporated to dryness in a rotary flash vacuum evaporator at reduced temperature. Paste from the extract obtained was further processed to obtain free flowing powder. 1000 g of *Garcinia cambogia* dried fruit rind was also extracted by using the same method.

Chemicals and Reagents

Orlistat, crystal violet, uric acid and ursolic acid were purchased from Sigma Aldrich Co., USA. Other analytical grade chemicals, reagents and solvents were purchased from HiMedia Laboratories, Mumbai, India.

Phytochemical study

The extracts of *Clerodendrum phlomidis* and *Garcinia cambogia* were subjected to preliminary analysis of the following phytochemicals according to the standard methods [6, 7, 8, 9, 10].

1. **Saponins**: 300 mg of extract was boiled with 5 ml of water for two minutes. The mixture was cooled and mixed vigorously and left for three min. The formation of frothing indicated the presence of saponins.

2. **Tannins**: To an aliquot of the extract, sodium chloride is added to make to 2% strength. Then it is filtered and mixed with 1% gelatin solution. Precipitation indicated the presence of tannins.

3. **Triterpenes**: 300 mg of extract was mixed with 5 ml of chloroform and warmed for 30 min. The chloroform solution was then treated with a small volume of concentrated sulphuric acid and mixed properly. The appearance of red color indicated the presence of triterpenes.

4. **Alkaloids**: 300 mg of extract was digested with 2 M HCl. Acidic filtrate was mixed with amyl alcohol at room temperature, and examined the alcoholic layer for the pink colour development, which indicated the presence of alkaloids.

5. **Flavonoids**: 300 mg of the extract was dissolved in 2 ml of 1% aluminum chloride solution in methanol, concentrated HCl, magnesium turnings, and potassium hydroxide solution were added and mixed. The presence of flavonoids was indicative if pink or magenta – red colour developed within 3 min.

6. **Glycosides:** 500 mg of extract was dissolved in 2.0 ml of glacial acetic acid containing one drop of FeCl3 Solution. This was then under laid with 1.0 ml of concentrated H_2SO_4. A brown ring appeared at the interface indicated the presence of glycosides.

7. **Phenols:** 500 mg of plant extract was dissolved in 2 ml of ethanol and treated with few drops of neutral ferric chloride solution 5%. Development of intense colour indicated the presence of phenols.

8. **Anthroquinones:** Detection of anthroquinones was done by using Borntreger's test. 1 g of plant extract was shaken with 10 ml of benzene. This was filtered and 5.0 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the appearence of violet colour in the ammoniacal (lower) phase indicated the presence of free hydroxyl anthroquinones.

9. **Terpenoids:** 500 mg of plant extract was dissolved in 2 ml of ethanol and taken in a test tube and then added few pieces of tin and 3 drops of thionyl chloride. Development of violet or purple colour indicated the presence of terpenoids.

10. **Steroids:** (Liebermann Burchard reaction): 200 mg of plant extract was dissolved in 10

ml of chloroform and filtered. 2 ml of filtrate + 2 ml of acetic anhydride + conc. H_2SO_4. were added. Appearance of blue green ring indicated the presence of steroids.

Quantitative Estimation of Phytochemicals

Estimation of Total Phenolics

The total phenolic content was determined according to the method described by Siddhuraju and Becker [11]. 10µl of extract samples were taken in test tubes and made up to 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu phenol reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and then read at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as gallic acid equivalents per 100 mg of dry weight.

Estimation of Total Tannins

Using the same extract, the tannin content was estimated after treatment with polyvinylpolypyrrolidone (PVPP) [12]. 100µg/ml of PVPP was taken in test tube and to this 1ml distilled water and then 1 ml sample extract were added. The content was vortexed and kept in the test tube at 4°C for 4 h. Then the sample was centrifuged (3000 rpm for 10 min) and the supernatant was collected. This supernatant has only simple phenolics other than tannins (the tannins would have been precipitated along with the PVPP). The free phenolic content of the supernatant was measured and expressed as tannic acid equivalents. From the above results, the tannin content of the sample was calculated as follows: Tannin (%) = Total phenolics (%) – Non-tannin phenolics (%)

Estimation of Total Flavonoids

The content of total flavonoids was determined by slightly modified colorimetric method described by Zhishen *et al* [13]. A 0.5ml aliquot was mixed with 2ml of distilled water and subsequently with 0.15ml of 5% $NaNO_2$ solution. After 6 min, 0.15 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 min, and then 2 ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5 ml, and then it was thoroughly mixed and allowed to stand for another 15min. Absorbance of the reaction mixture was determined at 510 nm and the results were expressed as rutin equivalent per 100 g of dry weight.

Estimation of Total Alkaloid Content

The total alkaloid contents in the samples were analyzed using 1, 10-phenanthroline by the method described by Singh *et al* [14] with slight modifications. 100 mg sample was extracted in 10 ml of 80% ethanol. This was filtered through muslin cloth and centrifuged at 5000 rpm for 10 min. Supernatant obtained was used for the further estimation of total alkaloids. The reaction mixture contained 1ml of extract, 1ml of 0.025M $FeCl_3$ in 0.5M HCl and 1ml of 0.05M 1,10- phenanthroline in ethanol. The reaction mixture was incubated for 30 min in hot water bath with maintained temperature of 70 ± 2ºC. After cooling down to room temperature, the absorbance of red colour complex was measured at 510 nm against reagent blank. Alkaloid content was calculated with the help of standard curve plotted with colchicine. The values are expressed as g/100g of dry weight.

Estimation of Total Saponins Glycosides [10]

5 g of leaf extract was dissolved in 25 ml of 90% methanol and refluxed for 1 h. Filter the methanol and repeat the reflux two more times. Combine three extracts and distill off the methanol. To the soft extract 25 ml of petroleum ether was added and refluxed for ½ h. After cooling and discard the pet ether layer. 25 ml of ethyl acetate was added to the residue and reflux for ½ h and cooled and decanted the solvent. The residue left was dissolved in 25 ml of 90% methanol Filtered and reduced the methanol content to 5 ml. To this methanol portion 25 ml of solvent ether was added drop by drop with constant stirring to precipitate the total saponins. The precipitate was filtered or decanted off the solvent and dry the residue to constant weight.

Estimation of Ursolic Acid in *Clerodendrum Phlomidis* Leaf Extract

(Modified method of Albuquerque [15] and Gao *et al*. [16]

Sample Preparation

5 g of *Clerodendrum phlomidis* leaf extract was dissolved in 25 ml of 50% (v/v) methanol and heated for its complete dissolution. To this 75 ml of water wa*s* added and contents were mixed thoroughly. Then the reaction mixture was transferred to a round bottom flask and 10 ml of H_2SO_4 was added and reflux for 6-8 h. The reaction mixture was cooled and transferred into a separating funnel and then 25 ml of chloroform was added. The chloroform layer was allowed to separate and it was transferred to another separating funnel. The aqueous acidic layer was once again washed with 25 ml of chloroform. Both the chloroform washings (50 ml) were combined and washed with water till it was acid free. By the addition of anhydrous

sodium sulphate, the water residue in the acid free chloroform layer was removed and after filtration, the chloroform was evaporated to get the sample.

Quantitative Estimation

0.01 g of the sample was dissolved in 3 ml of absolute alcohol and detected in a UV spectrophotometer at 550 nm. Ursolic acid standard was dissolved in 3 ml of absolute alcohol to produce different concentrations (3.33, 6.67, 9.99, 13.32 and 16.65%) and detected in the same nm. Average mean value of three determinations was calculated. The amount of ursolic acid present in the sample was calculated by plotting the curve with standard curve.

Estimation of (-) Hydroxy Citric Acid sin *Garcinia Cambogia* Fruit Extract [17]

0.5 g of *Garcinia cambogia* extract was dissolved in 3 ml of water and pectin and other starchy materials were precipitated by adding 1 ml of alcohol. The clear filtrate was taken and passed through cation exchange resin (Amberlite IR120 H^+) to liberate free acids. The content of (-) citric acid was estimated by acid base titration method.

High Fat Diet Formula

Corn starch-15%, Sugar-27.5%, Lard oil-17.6%, Vitamin mixture-1%, Mineral mixture-3.5%, Casein-20%, Cellulose powder-5%, corn oil-9.9%, Choline bitartrate- 0.2% and Methionine-3g which will be given along with normal pellet chow [18, 19].

Experimental Animals

Healthy adult female Wistar strain of albino rats, 4 months old and weighing 175-200g were obtained from Tamil Nadu Veterinary and Animal Sciences University, Chennai. The animals were allowed to acclimatize under standard laboratory conditions day / night rhythm (8.00 am 8.00 pm) 22±2^{0}C and RH 55±5% with a 12:12 light and dark cycle in polypropylene cages for a period of 5 days prior to the experiment. Animals were fed with standard rat chow pellet and provided water *ad libitum.* All procedures involving laboratory animal use were in accordance to the Institute Animal Ethics Committee regulations approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (Approval No: 790/03/ac/CPCSEA). The rats were divided into 5 groups, each group comprising of 6 rats. The total experimental period was 50days. Rats in Group I were fed with normal chow and water for 50 days. Rats in Group II, III, IV, and V were fed with high fat diet for 50 days and drug treatment started on 16th day of the experiment.

Group I: Normal healthy control animals, fed with Normal chow and water for 50 days.

Group II: Obesity induced control animals, received only high fat diet (HFD) for 50 days.

Group III: Obesity induced rats treated with high fat diet + *Clerodendrum phlomidis* leaf extract, 400mg/kg-bw/day orally from 16 to50th day.

Group IV: Obese induced rats treated with high fat diet + *Garcinia cambogia* fruit extract, 400mg/kg-bw/day orally from 16 to50th day. .

Group V: Drug control-animals, obese induced rats treated with high fat diet + standard drug Orlistat 32 mg/kg-bw/day orally [20] from 16 to50th day.

Approved medications for long term use in the treatment of obesity are Sibutramine and Orlistat. Sibutramine is an anorectic or appetite suppressant (reducing the desire to eat), whereas Orlistat reduce the fat absorption by inhibiting gastrointestinal lipase activity. Actually Orlistat is the only antiobesity drug allowed by FDA and also in Europe, because it is safe and well tolerated [20]**.** Hence Orlistat was used as standard drug in the present study.

Pharmacological Studies

Body Weight: The body weight (g) was recorded on day one and then on every week for 35 days using digital weighing balance.

Body temperature

The body temperature was recorded on 35th day by using rectal telethermometer before and after drug administration at 30, 60, 90, 120, 180 min with a contact time of 1 min.

Behavioral Activity

The exploratory behavior activity was recorded once in 5 days for 30 min after initiation of treatment using open field behaviour test apparatus for the treatment animals. The apparatus consists of fabricated chess board [black and white squares (5x5 cm)] in a circular wooden arena of 75 cm diameter and a wall with a height of 25 cm. Open field test were performed by placing the rat in the centre circle and recorded the ambulatory activity (squares crossed by horizontal movement). The frequency of rearing (standing up vertically) and grooming (face washing and repetitive licks directed to body) for a 5 min test period were recorded.

Weight of Organs and White Adipose Tissue [21]

The rats were sacrificed by cervical decapitation on 36th day. Then various organs, such as kidneys (right and left), liver, heart, and body fat pad of white adipose tissues (WAT)

periovarian, perirenal, mesenteric fat pad were isolated, rinsed in cold saline, dried on a filter paper and weighed. The organ to body weight (mg/g) ratio was recorded.

Biochemical Studies

Collection of Blood for Estimation of Biochemical Parameters

The blood was collected from the rat tail vein for the estimation of blood sugar by using glucometer and blood glucose test-strips, supplied by Ascensia Entrust of Bayer Health Care, Mumbai, India. For estimation of other biochemical parameters, blood was drawn from the retro-orbital plexus of the rats (fasted for 12 h), into sterilize eppendorf tubes. The blood samples were allowed to coagulate for 30 min at room temperature and then they were centrifuged at 3000 rpm for 10 min. The serum used as sample, should be free from haemolysis and must be separated from the clot promptly. The resulting upper serum layer was collected in the properly cleaned, dried, and labeled eppendorf tubes and they were stored at -8°C for further analyses.

Blood Glucose

Fasting blood glucose was estimated by using a commercial glucometer and test strips Accucheck Sensor test meter.

Estimation of Total Protein in Serum

Protein content was estimated by using Biuret reagent [22]**.** In a test tube 0.5 ml of plasma was mixed with 1.5 ml of 0.85% sodium chloride solution and 8 ml of Biuret reagent was added to it. To prepare standard, 1 ml of standard protein (Bovine serum albumin) was mixed with 1 ml of 0.85% sodium chloride solution. Then 8 ml of Biuret reagent was added to this. To prepare the blank 2 ml of 0.85% sodium chloride solution was mixed with 8 ml of Biuret reagent. All the test tubes were shaken well and allowed to stand for 30 min. Read the test and standard samples against blank at 520 nm.

Estimation of Free Fatty Acids in Serum [23]

0.1 ml of lipid extract was evaporated to dryness. 0.1 ml of phosphate buffer, 6.0 ml of extraction solvent (chloroform: methanol - 5:1) and 2.5 ml of copper reagent were added. All the tubes were shaken vigorously and then 200 mg of activated silicic acid was added and left aside for 30 min. The tubes were centrifuged and 3 ml of the upper layer was transferred to another tube containing 0.5 ml of diphenylcarbazide solution and mixed carefully. The absorbance was read at 550 nm. Palmitic acid was used as standard.

Extraction of Total Lipids in Serum [24]

5 ml of serum sample and 10 ml of chloroform was shaken well and filtered through Whatman No.1 filter paper. To the filtrate 2 ml of 0.9% NaCl solution was added and this reaction mixture was transferred into a separating funnel and it was allowed to stand overnight at 4°C. A clear biphasic layer was formed and lower phase containing all the lipids. The lipid layer was separated and the volume was made up to 10 ml by the addition of chloroform. This was transferred to a 50 ml beaker and the solvent was allowed to evaporate at 50-60°C for 5 h. Then 5 ml of concentrated $H_2SO_4$was added to it, mixed well, placed in a boiling bath for 10 min and was then cooled to room temperature. 0.2 ml of this was taken in a test tube and 5 ml of phosphovanillin reagent was added, mixed well and allowed to stand for 30 min. Standard was prepared by mixing 0.2 ml of standard cholesterol and 5 ml of phosphovanillin reagent, mixed well and allowed to stand for 30 min. Blank was prepared by adding 0.2 ml of chloroform to 5 ml of phosphovanillin reagent. Read test samples and standard against blank at 520 nm. The content of total lipids was expressed as µg/mg.

Estimation of Uric Acid In Serum [25].

Determination of uric acid concentration was measured by the direct enzymatic method, in which uric acid was oxidized by uricase coupled with peroxidase and results were measured in a colorimeter. Pipette 50 µl of plasma, standards, or reference sera into 0.5 ml of trichloroacetic acid (7.5 g/dl) in a test tube. Mixed the reaction mixture well and warm at 55-60ºC for 10 min, cooled to room temperature (20-25ºC), add 2 ml of borate buffer, and centrifuge. To 2.0 ml of the supernatant add 2.5 ml of buffered uricase (2.5 units/mg in 125 ml borate buffer), and left the mixture stand at room temperature for 15 min. Add 0.5 ml of working crystal violet solution (1M crystal violet in acetic acid), and 0.5 ml of peroxidase (10 mg of horseradish peroxidase in 100 ml of 40% glycerol in phosphate buffer). Allow to stand at room temperature for 15 min, then measure the absorbance at 590 nm within 30 min and water was used as blank. Also measure reagent blank, substituting water for serum and subtracts this value from the others. The uric acid was expressed as mg/dl.

Estimation of Serum Cholesterol [26]

To 0.1ml of serum 10 ml of ferric chloride reagent was added in a test tube. Mixed well and kept for 10 min at room temperature. It was then centrifuged at 3000 rpm for 30 min. 5 ml of the supernatant was pipette out into a test tube and 3 ml of concentrated sulphuric acid was added and mixed well. To prepare standard, 10 ml of working standard (200 mg of

cholesterol dissolved in 10 ml of ferric chloride reagent and made up to 100 ml with glacial acetic acid) was mixed with 0.1 ml of sodium chloride and kept for 10 min and centrifuged. 5 ml of supernatant was taken and to this 3 ml of concentrated sulphuric acid was added. Both the tubes were kept for 30 min at room temperature. To prepare the blank, 5 ml of ferric chloride solution was mixed with 3 ml of concentrated sulphuric acid. This was kept for 30 min. Read the test samples and standard against blank at 560 nm.

Estimation of Triglycerol (TGL) [27]

0.1 ml of serum was added to 4 ml of isopropanol, mixed well and to this 0.4 g of alumina was added and shaken well for 15 min. Centrifuged at 2000 rpm for 10 min and then 2 ml of the supernatant was transferred to appropriately labeled tubes. The tubes were placed in a water bath at 65° C for 15 min for saponification after adding 0.6 ml of the saponification reagent (0.5g of potassium hydroxide dissolved in 6 ml of distilled water and then 44 ml of isopropanol was added to it). After cooling down to room temperature 1 ml of sodium metaperiodate regent followed by 0.5 ml of acetyl acetone reagent were added. After mixing, the tubes were incubated in a water bath at 65°C for 30 min. The contents were cooled and read at 430 nm. Against appropriate blank. Tripalmitin (10 mg of tripalmitin was dissolved in 100 ml of isopropanol) was used as standard.

Estimation of HDL Cholesterol [28]

To 1.0 ml of lipid extract, 0.18 ml of heparin- manganese chloride reagent (3.167g of manganese chloride was added to 1 ml solution of heparin containing 20,000 units. The mixture was made up to 8 ml with distilled water) was added and mixed. This was allowed to stand in an ice bath for 30 min and then centrifuged in a refrigerated centrifuge at 2500 rpm for 30 min. The supernatant contained HDL fraction. Aliquots of the supernatant were estimated for LDL and HDL cholesterol and phospholipids.

Aggregation of VLDL

1 ml of lipid extract was added to 0.15 ml of SDS solution (sodium dodecyl sulphate - 10% in 0.15 M NaOH (pH-9). The contents were mixed well and incubated at 37°C for 2 h. The reaction mixture was centrifuged in a refrigerated centrifuge at 10,000 rpm for 30 min. VLDL aggregated as a pellicle at the top. The supernatant was a mixture containing HDL and LDL fractions. The fractions of lipoproteins were assayed after heparin manganese chloride and SDS precipitation. The values are expressed in mg/dl plasma. After precipitation the cholesterol levels in supernatant was measured to get HDL cholesterol. SDS precipitated

VLDL and the cholesterol content in the supernatant was measured for HDL cholesterol, LDL cholesterol and VLDL cholesterol.

LDL cholesterol = (Total serum cholesterol) – (Total serum TGL) - (HDL Cholesterol) / 5
VLDL =Total Serum TGL / 5

Estimation of Aspartate Transaminase (AST) [29]

The assay mixture containing 1 ml of substrate (1.33 g of aspartic acid and 15mg of keto glutarate dissolved in 100 ml of 0.1 M phosphate buffer (pH 7.5) containing 0.5mM NaOH) and 0.2 m1 of liver tissue homogenate was incubated for 1 h at 37°C. To the control tubes, liver tissue homogenate was added after the reaction was arrested by the addition of 1ml of 0.02% DNPH. The tubes were kept at room temperature for 30 min added 0.5 m1 of 0.4 N NaOH and the colour developed was read at 540nm. The activity of AST was expressed as moles of pyruvate formed/min/mg of protein.

Estimation of Alanine Transaminase (ALT) [29]

The assay mixture containing 1 ml of substrate (l.78 g of alanine and 30 mg of keto glutarate were dissolved in 100 ml of phosphate buffer containing 0.5mM NaOH) and 0.2 ml of liver tissue homogenate was incubated for 1 h at 37°C. To the control tubes serum was added after the reaction was arrested by the addition of 1ml of 0.02% DNPH. The tubes were kept at room temperature for 30 mm. added 0.5 ml of 0.4N NaOH and the colour developed was read at 540 nm. The activity of ALT was expressed as moles of pyruvate formed/min/mg of protein.

Estimation of Serum Alkaline Phosphatase (ALP) [29]

The reaction mixture containing 1.5 ml 0f of 0.1M carbonate buffer (pH 10), 1 ml of 0.1M disodium phenyl phosphate, and 0.1 ml of 0.1M magnesium chloride and 0.l ml of liver tissue homogenate was incubated at 37°C for 15 min. The reaction was arrested by the addition of Folin's Ciocalteau phenol reagent Control tubes were also treated similarly but serum was added after the reaction was arrested with Folin's Ciocalteau phenol reagent. Finally 1 ml of 15% Sodium carbonate was added. The colour developed was read after 10 min at 640 nm. The activity of
ALP was expressed as moles of phenol liberated/min/mg of protein.

Statistical Analysis

The data of results obtained were subjected to statistical analysis and expressed as mean ± SD. The data were statically analyzed by one way analysis of various (ANOVA) and to compare the means of the studied groups with *post hoc* Duncan multiple range tests at 5% and 1% for those results where significant difference was indicated. Values are expressed as mean±S.E.M (n=6) *p<0.05, **p<0.01, ***p<0.001 Compared with control

RESULTS

Table 1: Qualitative analysis of phytochemicals in *Clerodendrum phlomidis* leaf extract and *Garcinia cambogia* fruit extract.

Pytoconstituents	Clerodendrum phlomidis	Garcinia cambogia
Alkaloids	++	-
Flavonoids	++	++
Glycosides	++	++
Phenolic compounds	++	+
Phytosterols	++	+
Saponins	++	+
Tannins	++	++
Triterpenoids	++	++

‘+’Present, ‘-’ Absent; +, ++, +++ based on the intensity of the spots

*Values are means of triplicate determination.

TABLE 2: Quantitative analysis of phytochemicals in *Clerodendrum phlomidis* leaf extract and *Garcinia cambogia* fruit extract.

Chemical constituent	Clerodendrum phlomidis	Garcinia cambogia
Total phenolic compounds[1]	5.56±0.61	6.37±0.76
Total flavonoids[2]	0.62 ±0.96	0.58±0.66
Total alkaloids[3]	0.86±0.46	--
Total saponins	0.18% w/w	0.10% w/w
(-) hydroxy citric acid	--	6.15%
Ursolic acid	5.21%	--

1. g gallic acid equivalents/ 100 g dry wt of plant tissue
2. g rutin equivalents/ 100 g dry wt of plant tissue
3. g/ 100 g

TABLE -3: Effect of *Clerodendrum phlomidis* leaf extract and *Garcinia cambogia* fruit extract on body weight (g) in experimental rats.

Treatment Days	Weight of the animal in g (average of 6 rats)				
	Group I	Group II	Group III	Group IV	Group V
Initial	151.66±4.19	155.50±9.01	152.95±6.01	153.01±8.11	153.78±7.13

After obesity induction- 0 day	152.56±0.39	172.89±8.23**	174.86±7.91	173.12±8.39	174.25±8.16
7th Day	154.01±9.12	181.89±8.23***	173.71±8.34**	172.63±9.33**	174.46±8.21**
14th day	155.56±6.53	197.93±8.75***	169.57±9.38**	167.09±9.05**	161.24±8.22***
21rd day	156.91±8.55	209.57±0.13***	165.78±7.11**	148.38±7.09***	149.00±7.12***
28th day	157.56±6.53	217.93±9.75***	149.57±6.38***	147.09±6.05***	151.24±7.22***
35th day	158.91±6.55	224.57±10.13***	145.78±8.11***	144.38±6.09***	148.06±6.12***
Wt gain/ reduction	+5.44±0.09	+51.68±2.09***	-29.08±0.19***	-28.74±0.59***	-26.19±0.08***

Values are expressed as mean ± S.E.M (n=5) *p<0.05, **p<0.01, ***p<0.001 as compared with control group.

TABLE-4: Effect of *Clerodendrum phlomidis* leaf extract and *Garcinia cambogia* fruit extract on body temperature (ºC) of the experimental rats on 35th day of drug treatment.

Time in min	Body temperature (ºC)				
	Group I	Group II	Group III	Group IV	Group V
Before drug administration 30	32.5±1.02	32.5±1.08	33.7±1.10	33.0±1.13	32.2±1.08
After drug administration 0	33.1±1.12	33.0±1.02	32.0±1.10	33.1±1.14	33.2±1.12*
60	33.3±1.14	35.1±1.01*	37.8±1.11*	36.8±1.08*	36.9±1.07*
120	33.2±1.10	36.1±1.13*	38.1±1.11**	38.4±1.09**	37.7±1.12**
180	33.8±1.15	37.1±1.12*	38.9±1.07**	38.5±1.05**	38.5±1.10**

Values are expressed as mean ± S.E.M (n=5) *p<0.05, **p<0.01, ***p<0.001 as compared with control group.

TABLE- 5: Effect of *Clerodendrum phlomidis* leaf extract and *Garcinia cambogia* fruit extract on open field behaviour in experimental rats.

Behaviour	Frequency of open field behavior				
	Group I	Group II	Group III	Group IV	Group V
Ambulation (10 min)	76.4±5.13	60.8±2.12**	90.1±4.98**	94.0±3.71**	79.8±1.12**
Rearing (5 min)	42.4±2.12	22.4±1.41***	34.0±2.73**	34.0±1.37**	38.6±1.32**
Grooming (5 min)	12.4±0.84	6.4±0.85***	12.8±0.95**	14.5±0.60***	19.4±0.91***

Values are expressed as mean ± S.E.M (n=5) *p<0.05, **p<0.01, ***p<0.001 as compared with control group.

TABLE-6: Effect of *Clerodendrum phlomidis* leaf extract and *Garcinia cambogia* fruit extract on weight of organs in experimental rats.

Organ Wt.		Weight of the organ (g)				
		Group I	Group II	Group III	Group IV	Group V
Kidney	Left	0.57±0.11	1.15±0.17**	0.58±0.23**	0.66±0.10**	0.68±0.02**
	Right	0.55 ±0.19	1.30±0.12**	0.56±0.03**	0.64±0.09**	0.66±0.05**
Liver		5.10±0.21	17.88±0.24***	5.22±0.35*	5.05±0.36***	5.17±0.11**

Heart	0.50±0.43	1.52±0.04***	0.57±0.22***	0.58±0.18***	0.59±0.16***
Spleen	0.61±0.01	0.97±0.15***	0.65±0.13**	0.63±0.05**	0.61±0.12***

Values are expressed as mean ± S.E.M (n=5) *p<0.05, **p<0.01, ***p<0.001 as compared with control group.

TABLE- 7: Effect of *Clerodendrum phlomidis* leaf extract and *Garcinia cambogia* fruit extract on weight of fat pads in experimental rats.

Fat Pad	Fat Pad Weight				
	Group I	Group II	Group III	Group IV	Group V
Mesenteric	0.28 ± 0.12	0.92 ± 0.26***	0.78±0.17*	0.76 ± 0.89*	0.89 ± 0.27*
Periovarian	0.40 ± 0.03	0.87 ± 0.10***	0.56 ± 0.66**	0.53 ± 0.79**	0.71 ± 0.19*
Perirenal	1.29 ± 0.27	5.71 ± 0.35***	1.81 ± 0.52***	1.98 ± 0.17***	1.78± 0.21***

Values are expressed as mean ± S.E.M (n=5) *p<0.05, **p<0.01, ***p<0.001 as compared with control group.

TABLE- 8: Effect of *Clerodendrum phlomidis* leaf extract and *Garcinia cambogia* fruit extract on biochemical parameters in experimental rats.

Parameters	Experimental animals				
	Group I	Group II	Group III	Group IV	Group V
Blood glucose (mg/dl)	67.76±2.62	117.71±3.25***	84.40±0.10***	81.2±0.81***	80.61±0.10***
Total proteins (mg/g)	7.29±0.27	5.71±0.35***	6.81±0.54**	6.98±0.17**	6.78±0.23*
Total lipids (mg/g)	61.54±1.45	179.65±1.56***	68.70±1.89***	60.33±0.84	65.87±8.73*
Free fatty acids (mEq/L	0.763±0.01	1.461±0.11***	0.924±0.03**	0.943±0.05**	0.873±0.04**
Uric acid (mg/dl)	4.86±0.05	8.92±0.07***	5.65±0.09***	5.96±0.07***	5.96±0.08***

Values are expressed as mean ± S.E.M (n=5) *p<0.05, **p<0.01, ***p<0.001 as compared with control group.

TABLE-9: Effect of *Clerodendrum phlomidis* leaf extract and *Garcinia cambogia* fruit extract on liver function test in experimental rats.

Parameter	Experimental animals				
	Group I	Group II	Group III	Group IV	Group V
AST (U/L	28.04 ± 2.12	84.89 ± 2.26***	26.66±1.17***	28.23 ± 1.89***	29.11 ± 1.27***
ALT (U/L	33.40 ± 2.03	61.23 ± 3.10***	45.12 ± 1.66***	41.53 ± 1.79***	39.71 ± 1.19***
ALP-(U/L) (Alkaline phasphatase)	35.29 ± 3.27	49.71 ± 3.35***	38.81 ±1 .52***	36.98 ± 2.17***	36.73± 2.21***

Values are expressed as mean ± S.E.M (n=5) *p<0.05, **p<0.01, ***p<0.001 as compared with control group.

TABLE-10: Effect of *Clerodendrum phlomidis* leaf extract and *Garcinia cambogia* fruit extract on lipid profile in experimental rats.

Parameter	Experimental animals				
	Group I	Group II	Group III	Group IV	Group V
Serum Cholesterol (mg/dl)	83.82±2.50	139.20±11.54***	92.02±8.11**	90.43±9.31**	99.64±8.05***
SerumTriglycerides (mg/dl)	79.50±5.33	114.62±15.12**	82.33±8.12**	78.21±7.32**	80.43±6.45***
LDL-C (mg/dl)	44.42±0.06	78.03±2.54**	48.14±1.83**	46.78±2.90**	45.88±1.12***
VLDL-C (mg/dl)	34.31±1.61	51.06±0.99***	45.09±2.51**	42.79±0.88***	40.11±1.01**
HDL-C (mg/dl)	65.12±2.07	41.56±2.08**	60.30±1.02**	2.05±0.11**	64.19±0.80**
LDL/HDL ratio	0.68	1.87	0.79	0.75	0.72

Values are expressed as mean ± S.E.M (n=5) *p<0.05, **p<0.01, ***p<0.001 as compared with control group.

RESULTS

Obesity is a chronic and stigmatized disorder and it is more prevalent in developing/ developed countries and leading to much co-morbidity. Multiple factors such as environmental, metabolic and genetic and their interaction with each other regulate the body weight. Imbalance in either of the factors may be responsible for the development of weight gain/ obesity. Plant based therapeutics are much sought after drugs which play an important role in antiobesity and its pathogenesis. In the present study *Clerodendrum phlomidis* leaf extract was evaluated for its antiobesity activity and to compare its activity with a known antiobesity herbal drug, *Garcinia cambogia* fruit extract, and also with a standard drug, Orlistat.

Acute Toxicity Study

The acute oral toxicity studies of extracts were carried out as per the acute toxicity study method (**OECD** guidelines 425, 2001) using albino rats. Animals were kept fasting for overnight providing only water. The extracts of *Clerodendrum phlomidis* and *Garcinia cambogia* were suspended in 1% *Acacia* solution and administered stepwise doses from 100 mg/kg-bw up to the dose of 2000 mg/kg and observed the signs of toxicity up to 72 h in the tested animals [5]. The results clearly indicated non-toxicity of the extracts at a dose of 2000 mg/kg. From this, 1/5th (400 mg/kg- bw) dose was selected for the experimental study. Hence there was no LD50 and the extracts tested were considered as safe and nontoxic.

Phytochemical Analyses

Phytochemical analysis showed that alkaloids, flavonoids, glycosides, phenolic compounds, phytosterols, saponins, tannins and triterpenoids were present in the extract of both *Clerodendrum phlomidis* and *Garcinia cambogia* (Table 1). The quantity of phyto-constituents present in the extracts of *Clerodendrum phlomidis* and *Garcinia cambogia* are mentioned in Table 2. It was observed that 5.25% ursolic acid and 6.15% (-) hydroxy citric acid were quantified in *Clerodendrum phlomidis* leaf extract and *Garcinia cambogia* fruit extract respectively as active phytochemicals.

Effect on Body Weight

Obesity was induced in the experimental animals (Group II, III, IV & V) by feeding with High Fat Diet (HFD). Group II disease control animals showed significant ($p<0.001$) increase in body weight between day 1and day 50 when compared to group I healthy control animals. Treatment with *Clerodendrum phlomidis* leaf extract and *Garcinia cambogia* fruit extract showed a significant ($p<0.001$) decrease in body weight as compared with group II disease control (obese) animals. The rats treated with reference drug, Orlistat also showed significant ($p<0.001$) reduction in their body weight (Table: 3)

Effect on Body Temperature

Body temperature is the indication of fat utilization during thermogenesis which was well pronounced in plant drugs and standard drug treated animals. Animals, treated with *Clerodendrum phlomidis* leaf extract and *Garcinia cambogia* fruit extract and standard drug showed significant ($p<0.001$) increase in body temperature at 30, 60, 120 min when compared with healthy control animals (Table: 4).

Effect on Behavior Activity

There was a significant ($p<0.001$) decrease in ambulatory, rearing and grooming activity in HFD fed obese animals than healthy control animals. Treatment with *Clerodendrum phlomidis* extract and *Garcinia cambogia* extracts resulted in significant ($p<0.01$) enhancement in all the three activities (Table: 5).

Effect on Weights on Organs and Fat Pads

Group II animals fed with HFD showed significant ($p<0.01$) increase in their weight of the organs such as liver, spleen and heart and weight of mesenteric, periovarian and perirenal fat pads. Treatment with *Clerodendrum phlomidis* extract and *Garcinia cambogia* extract

resulted in significant ($p<0.01$) decrease in the weight of liver, spleen and heart and mesenteric, periovarian and perirenal fat pads (Table 6 and Table 7).

Effect on Biochemical Parameters

HFD had influenced to alter the levels of biochemical parameters such as blood glucose, total lipids, free fatty acids and uric acid. These parameters significantly ($p<0.01$) increase in obese induced Group II rats than healthy control rats of Group I rats. Animals treated with *Clerodendrum phlomidis* extract and *Garcinia cambogia* extract showed significant ($p<0.01$) increase in the level biochemical parameters investigated. The level of total protein was significantly ($p<0.01$) decreased in the obesity induced rats, but the treatment with plant drugs improved the level total protein (Table-8).

Effect on Liver Function Tests

The levels of AST, ALT and ALP were significantly ($p<0.01$) increased in obese induced group II rats than healthy control rats of group I rats. The rats treated with *Clerodendrum phlomidis* extract and *Garcinia cambogia* extract showed significant ($p<0.01$) decrease in the level of AST, ALT and ALP than obese induced rats. The rats treated with standard drug also showed significant decrease in the liver function biomarkers (Table: 9).

Effect on lipid Profiles

High fat diet induced obese rats (Group II) exhibited significant ($p<0.001$) increase in the lipid profiles such as blood glucose, total cholesterol, triglycerides, LDLc, and VLDLc when compared to healthy control (Group I rats). Obese rats after co-treated with plant drugs (Group III & IV) and standard drug (Group V) exhibited a significant ($p<0.01$) decrease in blood glucose, total cholesterol, triglyceride, LDLc, and VLDLc levels when compared to Group II animals. The Group II animals exhibited significant reduction in HDLc when compared to Group I animals. Group III, IV & V animals exhibited significant increase in HDLc level than Group II animals. LDLc/HDLc ratio also significantly ($p<0.01$) increased in obese induced rats when compared to the healthy rats. But the ratio was significantly ($p<0.01$) decreased after the treatment with both plant drug and standard drug treated rats (Table: 10).

DISCUSSION

Obesity is developed due to excessive calorie intake and sedentary lifestyle which result in major health problem [30] and obesity can be cured by inhibiting pancreatic lipase activity and

by inhibiting or delaying lipid and sugar absorption [31]. Dietary obesity can be readily induced in laboratory animals by giving HFDs. This shows that animals fed with HFD have higher energy intake than the normal diet group, and the HFDs contribute to the onset of obesity development of profound metabolic alterations [32, 33, 34]. Similar results are reported that consumption of cafeteria diet and atherogenic diet promotes obesity and fat accumulation in several animal species, including rats, mice, and pigs [35]. Phytochemicals are natural bioactive compounds present in the plants which are responsible for the therapeutic action of the plants in many disease conditions. The therapeutic constituents of *Clerodendrum phlomidis* leaf extract and *Garcinia cambogia* fruit extract are Ursolic Acid and (-) Hydroxy Citric Acid, respectively which are found to be useful in the treatment of obesity. The present study revealed that the hypolipidemic effects of *Clerodendrum phlomidis* leaf extract may be due to the presence of ursolic acid, and other phytochemicals such as phenolic compounds, flavonoids, alkaloids and saponins. It had been reported that (-) hydroxy citric acid has inhibitory action against pancreatic α-amylase and small intestine α- glucosidase enzymes [36] and there are reports that ursolic acid is having antilipidemic activity [37]. Another study reported that administration of *Sambucus australis* leaf extract containing 0.5% ursolic acid showed better results in the reduction of serum lipid levels in rats fed with cholesterol rich basal diet [38].

In the present study, the anti-obese activity of *Clerodendrum phlomidis* leaf extract and *Garcinia cambogia* fruit extract revealed that obesity induced by HFD elicited a significant increase in body weight, body temperature, organ and fat pad weights compared to healthy control. Obesity is considered to be a disorder of energy balance, occurring when energy expenditure is no longer in equilibrium with daily energy intake, so as to ensure body weight homeostasis[39]. Although the etiology of obesity is complex, dietary factors, particularly the consumption of HFD is considered as a risk factor for its development [40]. The current results showed that body weight increased significantly in the HFD fed rats compared with the normal diet fed rats. HFD acts as a source of saturated fat resulting in increase in body weight, levels of lipid profiles and blood glucose. Consumption of the HFD led to obesity because it facilitates the development of a positive energy balance leading to an increase in visceral fat deposition and this led to abdominal obesity [41]. In the current study, rats fed HFD were consumed considerably more amount of fat than the control rats throughout the experiment. So their calorie intake was increased and they showed an increase in perirenal visceral adipose tissue mass, suggesting that the excess energy led to the buildup of adiposity.

This is also the source for increasing the body weight. Rats consuming the high fat ration actually received about 27% more kilocalories, more weight, and had larger fat pads than rats fed only chow. The biochemical parameters such as blood glucose, total lipids, serum free fatty acids (FFAs) and uric acid salt levels were significantly increased in HFD fed obese rats and the levels were profoundly reduced in the rats treated with plant drugs. It is observed that hyperglycemia and hyperlipidemia in obesity are caused by insulin resistance and poor utilization of glucose and thus leads to the accumulated levels of blood glucose and total lipids. Serum FFAs play an important physiological role in skeletal muscle, heart, liver and pancreas. Further, in obese condition the excess FFAs is released from increased triglycerides by lipoprotein lipase and elevated level of FFAs in obese condition appears to cause lipotoxicity, which consequences the onset of peripheral and hepatic insulin resistance in all major insulin target organs like muscles, liver and endothelial cells[42]. This condition is further emerged as a major link between obesity and the development of oxidative stress, metabolic syndrome and atherosclerotic vascular disease. FFAs also produce low-grade inflammation in skeletal muscles, liver, blood vessels and adipose tissues which may contribute to cardiovascular events. It is opined that the near future challenges in obesity treatment must be included the correction of obesity related elevation of FFAs levels. In the present study it is proved that plant drugs have reduced the levels of FFAs in obesity induced rats.

The biochemical marker, uric acid is generated from purines which arise from the metabolism of dietary and endogenous nucleic acids through the action of the enzyme xanthine oxidase. Uric acid is a potent endogenous antioxidant, which scavenges the nitric oxide directly and thus decreases the bioavailability of nitric oxide in vascular smooth muscles and endothelial cells. Consequently this will cause endothelial dysfunction that enhances the risk of progression of cardiovascular disease. The higher concentration of uric acid present in the serum is known as hyperuricemia, which can cause serious health problems including renal insufficiency. Further, hyperuricemia is associated with many diseases including hypertension, diabetes mellitus and obesity. Gout is a painful rheumatic disease, resulting from the deposition of monosodium urate (micro crystals of uric acid salt) in various parts of the body, primarily in the joints and surrounding tissues due to overproduction or under excretion of poorly soluble uric acid. Inter-relationship between concentrations of serum uric acid and cardiovascular and cerebrovascular diseases has been reported for a long time[43,44,45]. In the present study the level of serum uric acid was elevated in the HFD

induced obese rats compared with healthy rats, but treatment with *Clerodendrum phlomidis* leaf extract and *Garcinia cambogia* fruit extract significantly decreased the uric acid level. Thus the plant drugs proved their anti- hyperuricemia effect.

The level of total proteins was decreased in rats fed with HFD when compared to rats with normal diet and same was brought back normalcy after the treatment with plant drugs. The decreased level of total proteins may be due to reduction in protein synthesis and reduced uptake of protein as a result of a high calorie lipid diet, an indication diminished synthetic function of the liver resulting probably from hepatocellular damage or stress resulting from the increased metabolic need for tissue repair and free radical neutralization due the high fat diet [46]. It is noteworthy that co-administration of plant drugs significantly restored the protein levels.The liver biomarker enzymes such as AST, ALT and ALP are the indicators of liver function. The levels of these enzymes were significantly increased in the obese rats when compared to healthy rats. The profound increase in the levels of biomarker enzymes indicates an impaired liver function. In obese condition the liver is bombarded by the free fatty acids that pour out of the adipose tissues into the portal blood. This can directly cause inflammation within the liver cells, which then release further pro-inflammatory cytokines, leading to more liver injury and affecting the integrity of liver cells [47]. The increased levels of biomarker enzymes and the formation of steatosis in obese rats correlate with the significant increase of liver weight. The present study demonstrated that animals treated with *Clerodendrum phlomidis* leaf extract and *Garcinia cambogia* fruit extract showed a profound decrease in the levels of AST, ALT and ALP. similar result was observed in the anti-obesity activity of *Lantana camara* plant extract on hyperlipidemia mice [48], and also our observation is in agreement with the result obtained by Celik and Isik[49] and El-Sayed Bakr and Eslam Header[50]. The treatment with *Clerodendrum phlomidis* leaf extract and *Garcinia cambogia* fruit extract resulted in the reduction of biomarker enzymes activity and liver weight in HFD fed obese rats indicating that the extracts possess hepatoprotective property. similar result were reported in the rats treated with ethanolic extract of *Bauhinia variegata* in HFD induced obese rats by Prashar and Venkatraman[51]. Dietary cholesterol was used in the HFD to induce obesity and the dietary cholesterol caused a significant elevation in the levels of lipid constituents in the serum such as total cholesterol, serum triglycerides, LDL cholesterol, and VLDL cholesterol and decreased the HDL cholesterol level. The elevated levels of serum lipid constituents increase the risk of atherosclerosis and coronary heart disease. Co-treatment of *Clerodendrum phlomidis* leaf extract and *Garcinia cambogia* fruit extract showed

significant decrease in the levels of atherogenic agents like serum cholesterol, triglycerides, LDL and VLDL cholesterols along with significant increase in protective agent, serum HDL cholesterol level. This trend was reflected in the LDL/HDL ratio. LDL/HDL ratio was markedly higher in obese rats than healthy control rats. After the treatment with plant extracts the ratio was brought back to normalcy in the ratio. HDL cholesterol is responsible for the mobilization of cholesterol from peripheral cells to the liver. Based on the results from the present study it can be considered that *Clerodendrum phlomidis* leaf extract and *Garcinia cambogia* fruit extract are having the cardioprotective efficacy. Total cholesterol and triglycerides are defined as the main risk factor for dyslipidemia [52] and the levels of both total cholesterol and triglycerides were significantly increased in HFD fed obese animals. After the treatment with plant drugs, the obese animals showed a significant decrease in the levels of lipid profiles. This results indicates the antihyperlipidemic potential of *Clerodendrum phlomidis* leaf extract and *Garcinia cambogia* fruit extract. Further, lipoproteins such as HDL, LDL and VLDL showed a profound increment in their production in obese rats, which were brought back to normal levels by the treatment of *Clerodendrum phlomidis* leaf extract and *Garcinia cambogia* fruit extract. Similar results were reported in obese rats treated with *Zizyphus jujube* leaf extract[53] *Bauhinia variegata* [54], *Commiphora mukul* [55] and *Zingiber officinale* [56], *Artemisia capillaries*[57] , *Momordica dioica*[58] and aqueous extract of green tea (*Camellia sinensis*)[59].

From the observations obtained from the present animal model study, it can be predicted that *Clerodendrum phlomidis* leaf and *Garcinia cambogia* fruit extracts showed significant anti-obese activity due to its hypoglycemic and hypolipidemic effect in rats fed with HFD. *Garcinia cambogia* fruit is a rich source of (-) hydroxyl citric acid, the active compound that aids in weight loss by inhibiting fat production and suppressing appetite[60]. Ursolic acid present in *Clerodendrum phlomidis* leaf extract is found to have weight loss activity by the inhibitory activity against pancreatic lipase. Other reports also support our claim that ursolic acid is a potent pancreatic lipase inhibitor [61, 62].

CONCLUSION

Based on these broad observations, we suggest that high fat diet-induced obesity resulted in deleterious effects in kidney and liver tissues. *Clerodendrum phlomidis* leaf and *Garcinia cambogia* fruit extracts administration counteracted the injuries, and ameliorated and/or normalized most of the biochemical parameters. Collectively obesity is associated with

metabolic complications, including insulin resistance, dyslipidemia, cardiovascular disease. When comparing therapies for obesity, both plant extracts have influenced the improvements in amelioration of body weight, levels of lipid profiles, markers of liver, kidney and cardiac function, as well as insulin resistance. So the plant extracts have hypolipidemic, renoprotective, hepatoprotective, antioxidant and antiobesity effect.

Further, it is proved from the present study that the antiobesity potential of *Clerodendrum phlomidis* leaf extract is comparable with *Garcinia cambogia* fruit extract and standard drug, Orlistat and it is concluded that *Clerodendrum phlomidis* leaf extract may be an efficient antiobesity agent. The pharmacologically active compound, ursolic acid and other phytochemicals present in the leaf may be responsible for the antiobesity activity and the effect may be partly through delaying the intestinal absorption of dietary sugar and fat by inhibiting pancreatic amylase and lipase activity. However, further experiments are required to understand the mechanisms involved in antiobesity activity by *Clerodendrum phlomidis* leaf extract.

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