ASSESSMENT OF THE ANTI-OBESITY EFFICACY OF ETHANOLIC EXTRACTS OF *SAUSSUREA LAPPA* (COSTUS) ROOTS IN CAFETERIA DIET FED OBESE RATS

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Abstract

Obesity and oxidative stress are two crucial risk factors that closely allied with metabolic syndrome. The prevalence of obesity and related diseases is rising worldwide, and there is a need for safe and effective treatments to combat this issue. The study investigated the phytochemical composition, total phenolic content (TPC), total flavonoid content (TFC) of the ethanolic root extract of *Saussurea lappa*. The phytochemical screening of the root extract revealed the presence of flavonoids, phenol, tannins, alkaloids, terpenoids and saponins. The TPC of the root extract was found to be 43.35±1.20 mg GAE/1000mg, while the TFC was found to be 37.56±0.52 mg QE/1000mg. Further study was explored effect of ethanolic extract of *Saussurea lappa* root on DPPH free radical scavenging, α-amylase, α-glucosidase and pancreatic lipase inhibition activity by using in vitro method. Our results revealed that IC50 values were found to be 121.94 µg/mL, 54.66µg/mL, 55.10µg/mL and 97.34µg/mL for DPPH free radical scavenging, α-amylase inhibition, α-glucosidase inhibition and pancreatic lipase inhibition respectively.

Quantitative and qualitative phytochemical screening also revealed the various phytoconstituents of *Saussurea lappa* root extract indicated promising natural ingredients to scavenge free radicals that may be associated with anti-obesity and anti-diabetic effects. The α-amylase, α-glucosidase and pancreatic lipase inhibition suggested that the root extract may have hypoglycaemic effect and also reduced the digestion of fats and triglycerides thus this plant may act as alternative herbal approach for management of metabolic syndrome.

The current study aimed to evaluate the anti-obesity activity of ethanolic root extracts of *Saussurea lappa* (Costus) roots in obese rats fed cafeteria diets. Male Wistar rats were divided into five groups: normal control, negative control, positive control and two experimental groups treated with different doses of the ethanolic root extracts. The negative control group (cafeteria diet fed) showed a significant increase in body weight, food intake, and lipid profile compared to the normal control group. However, treatment with ethanolic root extract of of *Saussurea lappa* (200mg/kg/day) resulted in a significant reduction ($P<0.05$) in body weight, food intake, anthropometrical measures, adipose tissue weight, adiposity index as well as various lipid profiles as compare to negative control group. These findings suggest that *Saussurea lappa* (Costus) root ethanolic extract may have potential anti-obesity effects and could be used as a safe and effective treatment for obesity and related metabolic abnormalities.

Keywords: Antiobesity, *Saussurea lappa*, Cafeteria diet, Metabolic syndrome

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INTRODUCTION

Since 1975, the frequency of obesity has almost tripled, making it possible to describe it as a global epidemic. One billion people worldwide are obese, including 650 million adults, 340 million adolescents, and 39 million children, according to a new World Obesity Federation (WOF) research[1,2]. The growing occurrence of obesity in children and teenagers raises severe concerns, since it increases the possibility that adult obesity may get worse in the future[3,4]. Obesity is characterized by low-grade inflammation due to increase in mass of adipose tissue causing hypoxia which in turn secretes pro-inflammatory cytokines[5]. Depending on its duration, the amount of extra weight, the dysregulation of metabolic homeostasis, and the development of related comorbid disorders such insulin resistance, dyslipidemia, hypertension, and others, obesity is allied to a 5–20 years reduction in life expectancy[6,7]. There are many ways to prevent or control obesity, which includes, diet regimes, exercise and medication[8,9]. However, the use of anti-obesity drugs such as orlistat, rimonabant and sibutramine has been reported to cause various adverse effects including constipation, dry mouth, headache, heart attack and insomnia[10]. Thus, there is a great demand for the search of new and safer anti-obesity agents. Moreover, numerous preclinical and clinical studies, various herbal plants that were reported significant improvement in controlling body weight without any minimal side effects[11]. So, herbal plants are a potential alternative treatment strategy for the development of effective and safe anti-obesity and anti-diabetes drugs.

Saussurea lappa C.B. Clarke (Syn: Saussurea costus) belonging to family Asteraceae, commonly-known as kuth or costus. It is one such indigenous herb naturally found at an altitudinal range of 2000-3500 m above mean sea level of the north western Himalaya and its neighboring areas[12]. *Saussurea* species have phytoconstituents such as sesquiterpenoids, lignans, flavonoids, and steroids[13]. Various therapeutic potentials of costus have been reported over the years in traditional as well as conventional medication system. The roots of *S. lappa* were prominent for their therapeutic characteristics, with antihelminitic, anti-epileptic, immunomodulatory, hepatoprotective, antioxidant, CVD and hyperlipidmic activity, Neuroprotective activity, anti-inflammatory, antilarvicidal, antifungal, anti-bacterial and anticancerous activity[14, 15].

The main factors contributing to the rise in obesity are changes in western-style diets brought on by changes in food availability, quality, amount, and source[16]. Researcher have suggested there are numerous techniques used to promote excess fat deposition, and diet-induced-obesity animal models can replicate human overweight and obesity[17,18]. Animals are given full access to a variety of tempting processed meals (such as cookies, cake, chips) and water, which was developed to closely resemble the feeding patterns of humans[19]. Depending on the strain and gender of the rodent and the source of dietary fat, studies have shown that a cafeteria diet can cause hyperphagia, weight gain, hyperleptinemic, hyperinsulinemic, hyperglycemic and hypertriglyceridemic[20,21]. This model with its physiological properties replicates many of the features observed in obese human and also mimics human obesity better when compared to the genetic model. However, its anti-obesity profile of *S. lappa* has not been documented. Therefore, the objective of the present study is to investigate the effect of the ethanolic extract of *S. lappa* roots on cafeteria diet induced obese rats.

MATERIAL AND METHODS

Chemicals and Reagents

Saussurea lappa was purchased from the local market, Indore (India), DPPH free radical, Folin-Ciocateu reagent, sodium carbonate, sodium acetate, aluminum chloride hexahydrate, quercetin, p-nitro phenyl palmitate, p-nitrophenyl α-D-glucopyranoside, lipase from porcine pancreas, isopropanol, Triton X-100, were purchased from Sigma-Aldrich. Ethanol, methanol, hydrochloric acid and gallic acid were obtained from were obtained from Qualigens Fine Chemical, India. Orlistat was obtained from Sun Pharmaceutical Ltd, India. Triglyceride GPO-PAP, Cholesterol CHOD-PAP, LDL, HDL cholesterol and Glucose GOD POD kits were procured from Erba mannheim ltd, India

Preparation of *Saussurea lappa* extract

The roots of *S. lappa* were purchased from the local market of Indore. The plant species was authenticated by a botanist of Janta PG College, Rewa, M.P., (Accession No. J/Bot/SLR-019). The roots were air dried, powdered, and then extracted with ethanol by using the Soxhlet method. The extract was filtered with Whatman No. 1 filter paper and then the solvent evaporated at a reduced pressure by using the Rotavapor apparatus to get a viscous mass, which was then stored at 4°C until used. In this way, 3.42g dried extract was prepared

from each of the 100g dried powder of *S. lappa* root. The ethanolic extract of *S. lappa* and orlistat were dissolved in distilled water and add tween 80 to prepare suspension.

Qualitative phytochemical screening[22, 23]

Standardized phytochemical testing procedures were followed to detect the active secondary metabolites in order to establish the chemical profile of plant for phenols, carbohydrates, flavonoids, alkaloids, terpenoids, saponin, tannins, glycosides etc

Quantitative phytochemical screening[24]

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminum chloride method. 10 mg Quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.1 ml of 2% AlCl3 solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

Estimation of total phenol content

The total phenol content of the extract was determined by the modified folin-ciocalteu method.10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/L) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

***In-vitro* antioxidant activity using DPPH method**[25]

The spectrophotometer was used to measure the DPPH scavenging activity. The stock solution (6 mg in 100 mL methanol) was produced to give an initial absorbance of 1.5 mL in 1.5 mL methanol. 1.5 ml of DPPH and 1.5 ml of varying concentrations of the standard and test sample were placed in a succession of volumetric flasks, and the final volume was adjusted to 3 ml using methanol. Three test samples were collected and processed in the same way. Finally, the average was calculated. After 15 minutes at 517 nm, the absorbance of DPPH with varied concentrations showed a final reduction.

$$Radical\ scavenging\ activity\ (\%) = (A_{control} - A_{test})/A_{control} \times 100$$

Where $A_{control}$ is the absorption (without extract) of the control and where A_{test} is the absorption in the presence of the extract / standard.

α-amylase inhibition assay[26]

The Alpha-amylase activity was estimated using method described by Xiao et al., 2006 17 with slight modification. The substrate was prepared by dissolving starch (500 mg) in 25 mL of 0.4M NaOH and boiling for 5min at 100 °C. The substrate (40 µl) and different plant extract in varying concentration were mixed and preincubated at 37 °C for 3 min. Then 20 µL of the α amylase solution (50µg/mL) was added to each well, and the plate was incubated for 15 min. The reaction was terminated by adding 80 µL of 0.1M HCl and then 200 µL of 1mM iodine solution was added. The absorbance (Abs) was measured at 650 nm. Inhibitory activity was calculated as follows:

$$Inhibition\ (\%) = (A_{control} - A_{test})/A_{control} \times 100$$

Where $A_{control}$ is the absorption (without extract) of the control and where A_{test} is the absorption in the presence of the extract / standard.

α-glucosidase inhibition assay[26]

The inhibition of α-glucosidase was measured using 1 mg of α glucosidase dissolved in 100 mL phosphate buffer pH 6.8 and 5mM p-nitrophenyl α-D-glucopyranoside. Plant extracts at different concentration were premixed with 490 µL phosphate buffer pH 6.8 and 250 µL of 5 mM p-nitrophenyl α-D-glucopyranoside (p-NPG). After pre-incubation at 37 ºC for 5 min, 250 µL α-glucosidase (0.15 unit /mL) was added and incubated at 37 ºC for 15 min. The reaction was terminated by the addition of 2000 µL Na2CO3 200 mM α-glucosidase activity was measured at 400 nm by monitoring the quantity of p-nitrophenol released from p-NPG at 400 nm. The concentration of the extract required to inhibit 50% of α-glucosidase activity under the assay conditions was defined as the IC50 value.

Pancreatic lipase inhibition assay[27,28]

The majority of triglyceride hydrolysis is done by pancreatic lipase and hence porcine pancreatic

lipase was used as an enzyme model. Crude lipase was dissolved in reaction buffer (10mg/ml) and centrifuged at 7000 rpm for 10min.Lipase assays were performed with 200µl reaction volume. P-NPP was used as a substrate with a reaction buffer of 50mM sodium phosphate, 5mM sodium deoxycholate and 10% iso-propranolol at pH 8.0. then increasing concentrations of *S. lappa* extracts and orlistat at 50, 100 and 150 and 200 µg/ml was mixed with 20µl of the enzyme buffer and incubated for 15 min at 37ºC with 5 µL of the substrate solution [10mM pNPP (p-nitro phenyl palmitate) in dimethyl formamide]. The enzymatic reactions were allowed to proceed for 30 min at 37ºC. Lipase activity was determined by measuring the hydrolysis of pNPP to p-nitrophenol at 405 nm using an ELISA reader. The Percentage inhibition (IA%) was calculated using the following equation:

$$Inhibitory\ activity\ (IA\ \%) = (A - B)/A \times 100$$

Where A is lipase activity in the reaction solution without sample and B is lipase activity in the reaction solution containing sample. The IC50 values were determined from the plots of percentage inhibition Vs concentration.

Experimental animals

Healthy male Wistar rats (80-100g) aged 7-8 weeks, was procured from animal facility, Acropolis College, Indore. Animals were housed in cages at a room temperature of 22 ± 2°C, humidity 55 ± 5% with a 12:12 hr light-dark cycle and had access to water and Normal pellet diet (Amrut Laboratory Animal Feed, India, protein 22.12%, fat 4.13%, carbohydrate) and Cafeteria diet ad libitum as per the study. All the study protocols were approved by Institutional Animal Ethics Committee (IAEC) and experiments were performed in accordance with the guidelines laid down by the Committee for Control and Supervision of Experimentation on Animals (CPCSEA). Effect of the *S. lappa* extracts were observed primarily on acute and chronic animal models for fat induced hypertriglyceridemia and obesity, respectively.

Acute toxicity studies[29]

Albino mice weighing 22-25 g selected by random sampling technique were used in the study. Acute oral toxicity was performed as per OECD- 423 guidelines. The animals were fasted overnight, provided only water after which extract was administered to the groups orally at the dose level of 5 mg/kg body weight by gastric intubation and the groups were observed for 48 hrs. If mortality was observed in 2 or 3 animals among 6 animals then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2,000 mg/ kg body weight. The animals were observed for toxic symptoms such as behavioral changes, motor reflexes and mortality for 48 hours.

Chronic Animal Model: Cafeteria diet induced obesity[30]

This model is fed to cafeteria diets in following manner: - Cafeteria diet was prepared using (a) Condensed Milk (8 g) + Bread (8 g). (b) Chocolate (3 g) + Biscuits (6 g) + Dried Coconut (6 g), and (c) Cheese/Vegetable oil (8 g) + Boiled Potato (10 g). The three diets were presented to the individual rats on days one, two, and three, respectively, and then repeated for 84 days in the same succession. The normal control group continued to be fed a laboratory normal pellet diet ad libitum.

Induction of obesity in the rats

Obesity was induced by feeding the experimental animals with a prepared cafeteria diet (CD) and water ad libitum for 12 weeks. The composition of diet was done according to formula described by Harris et al. with some modification. Rats with Lee obesity index value of 310 (equivalent to BMI in human) and above considered obese and used in the study.

Experimental Design

In cafeteria diet-induced model, After the period of obesity induction, the animals were divided into five groups and each group comprised of six animals. The first group fed on normal pellet diet consisted of normal rats for 12 weeks.

Animals were grouped as follows:

Group I: Vehicle-treated group (Normal control)
Group II: Cafeteria diet control (Negative control)
Group III: Cafeteria diet + Orlistat (30mg/kg/day, BW) (Positive control)
Group IV: Cafeteria diet + Ethanolic extract of *S. lappa* (100 mg/kg/day, BW)
Group V: Cafeteria diet + Ethanolic extract of *S. lappa* (200 mg/kg/day, BW)

All the treatments were carried out for 12th to 18th weeks. During the entire dosing period, all the treated rats were maintained on cafeteria diet. All the experiment rats received water ad libitum throughout the study period.

Food intake and body weight measurement

The daily food intake of the rats was measured in the morning using a weighing balance. Food intake was calculated by subtracting the amount of food left over in each cage (that is, the refusal and spillage for the individual solid diets) from the measured amount of food provided at the previous day (gm/day/cage). The mean of food intake was represented in gm/day/group. The body weight of the rat was measured weekly in grams (g) throughout entire duration of the study.

Anthropometrical determinations [31]

The anthropometric and morphological measures were determined once every week. The obesity index determined by Lee Index. The Lee index was calculated according to formula described by Lee.

Lee Index (%) = Cube root of Body Weight (g)/ Nose-to-Anus Length(cm) x1000

The body length (nose-to-anus length) was determined weekly in centimetre (cm) in all rats. The body length of rats was determined by a non-extensible thread and reading taken using a ruler with an accuracy of 0.1 cm. The body weight and body length were used to determine the body mass index as described by Noveli et al.

$$Body\ Mass\ Index = Body\ Weight\ (g)/BodyLength^2\ (cm^2)$$

Obesity was defined by Lee Index and BMI of greater than 310 and 0.67. Following exposure to CD (except for normal control) for 8 weeks, all the rats in the negative control, positive control and extract administered experimental groups attained the target diagnostic value of obesity, indicating the end of the obesity induction phase.

Abdominal circumference was assessed on the largest zone of the rat abdomen in front of the hindleg using a non-extensible thread. During entire period, the rats were placed in ventral position. The reading of abdominal circumference was taken using a ruler with an accuracy of 0.1 cm as described by Noveli et al.

Biochemical Analysis

At the end of the study, rats were fasted for 12 to 14 h. blood was collected by retro-orbital puncture from the ether-anesthetized rats. The blood samples were collected in plain tubes, allowed to coagulate at room temperature and centrifuged at 3500 rpm for 15 min at room temperature for separation of serum. The clear, non-hemolyzed supernatant separated using clean dry Pasteur pipette and stored at 4°C. The serum levels of glucose, total-cholesterol, HDL, LDL, and triglycerides (TGs) were estimated using the semi-auto analyzer (Microlab RX50V).

Estimation of liver & adipose tissues and adiposity Index

At the end of study rats were sacrificed by cervical dislocation. After abdominal incision, liver and white adipose tissues were harvested from each rat. The tissues were rinsed in cold normal saline, blotted and dried on filter paper and weighed (g). Adiposity index was determined by the sum of epididymal, visceral and retroperitoneal fat weights divided by body weight × 100, and expressed as adiposity percentage by Taylor et al.

Statistical Analysis

The results were expressed as mean ± standard error (SEM). The data were analyzed by one-way analysis of variance (ANOVA) followed with Tukey's multiple comparison, and $P<0.05$ was considered statistically significant by using GraphPad Prism 5.0 (La Jolla, CA, USA) software.

RESULTS

Phytochemical Screening

Phytochemical screening of *S. lappa* root ethanolic extract revealed the presence of flavonoids, terpenoids, saponin, glycosides, sterols, flavonoids, tannins, and phenolic compounds. Phytochemicals play a role in the treatment of obesity through various mechanisms. Preliminary phytochemical composition of various extracts of the *S. lappa* roots are following:

Table1:- Results of phytochemical Screening

Phytoconstituents	Ethanolic root extract of *S. lappa*
Flavonoids	+++
Phenol	++
Tannins	+
Terpenoids	+
Saponins	+
Alkaloids	+
Glycoside	-
Carbohydrate	-
Protein	-

The signs that indicate - Absent, + Present in Trace amount, ++ Moderately present, +++ Present in higher

Quantitative phytochemical screening

S. lappa showed highest polyphenolic content which is expressed as gallic acid equivalent. The

flavonoid content was expressed as quercetin equivalent *S. lappa* showed highest concentration of these phytochemicals compared to other plants are shown in

Table 2: - Quantitative phytochemical screening

Assay	**Ethanolic root extract of *S. lappa***
Total flavonoid content Quercetin eq. mg/1000mg	37.56±0.52
Total phenolic content Gallic acid Eq. mg/1000mg	43.35±1.20
DPPH free radical scavenging (IC50)	121.94 µg/mL
Alpha amylase Inhibition Assay (IC50)	54.66 µg/mL
Alpha Glucosidase Inhibition Assay (IC50)	55.10 µg/mL
Lipase Inhibition assay (IC50)	97.34 µg/mL

Effect of ethanolic root extract of *S. lappa* on DPPH free radical scavenging activity

The antioxidant activity was analyzed by an array of *In-vitro* assay *S. lappa* showed highest reducing power in comparison with other plants and the activity was expressed in ascorbic acid equivalent. There was dose dependent increase in quenching of free radical with the increase in extract concentration. The results are expressed as IC50 values are shown in Table 2. Ethanolic extract of *S. lappa* roots exhibited stronger antioxidant activity.

Effect of ethanolic root extract of *S. lappa* on α-amylase, α-glucosidase and pancreatic lipase inhibition activity

In this study, we investigated the inhibitory activity of ethanolic root extracts of *S. lappa* against α-amylase and α-glucosidase inhibition assay. As shown in table 2, the IC50 values of ethanolic extracts *S. lappa* root were found to be 54.66µg/mLand 55.10 µg/mL for α-amylase and α-glucosidase inhibition assay respectively, this result revealed that hypoglycemic potential of ethanolic extract of *S. lappa* root. Acarbose used as standard α-amylase/ α-glucosidase inhibitor as standard for comparison. Pancreatic lipase inhibitor activity was calculated using p-nitro phenyl butyrate as substrate and Orlistat as standard drug. There was dose dependent increase in inhibitory activity of lipase with increase with ethanolic extract of *S. lappa* concentration. This was expressed as IC50 value. Our result revealed that ethanolic extract of *S. lappa* root showed 97.34µg/mL thatindicated the strong inhibitory activity against pancreatic lipase.

Effect of ethanolic root extract of *S. lappa* on body weight of cafeteria diet indued obese rats

Table 3shows the changes in body weight in the different group of animals, during the experiment. Consumption of cafeteria diet for six weeks produced a significant ($P < 0.05$) increase in body weight compared to the consumption of normal pellet chow (normal control group). Treatment of rats with reference drug, Orlistat and the two extract doses caused a decrease in body weights from the first to the six weeks of the study periods. The rat models administered with ethanolic extract of *S. lappa* at a dose of 200 mg/kg showed negative changes in body weight from -3.35 % in the first week to -17.41% in the six weeks of study. At the end of study, the body weight of negative control group was significantly higher than those of extract treated rats, and Orlistat treated rats and rats in normal control group ($P < 0.05$). Meanwhile, rats treated with two extract doses recorded the reduction in the body weights relative to other experimental groups.

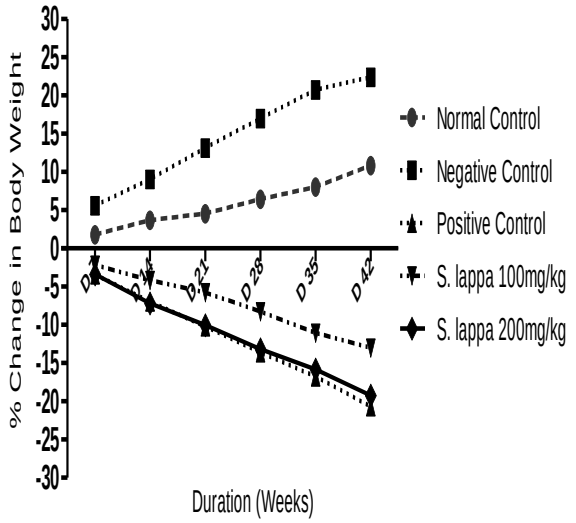

***Fig1.** The mean percentage change in body weight of experimental rats treated with the ethanolic root extract of Sausurea lappa for six weeks*

Table: -3 Effect of oral administration of ethanolic extract of *S. lappa* root for six weeks on body weight of cafeteria diet (CD)fed obese rats.

Treatments (mg/kg, BW)	Weekly Body Weights of Rats(g)						
	Day 1	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 35**	**Day 42**
Normal Control	132.50±	134.83±	137.33±	138.50±	141.00±	143.17±	146.83±
	1.06	1.12	1.27	1.30	1.21	0.99	1.08
Negative Control	203.50±	214.83±	221.83±	230.00±	238.00±	245.67±	249.00±
	3.29	2.41	2.28	2.48	2.21	1.96	1.99$^{\#\#}$
Positive Control	205.50±	198.50±	191.17±	184.50±	177.50±	171.00±	163.0±
	1.64	2.12	1.97	1.89	1.60	1.59	1.23***
CD + *S. lappa*100mg/kg	207.33±	202.83±	198.67±	195.33±	190.17±	184.33±	180.33±
	1.89	1.67	1.83	1.55	1.52	1.46	1.17**
CD + *S. lappa* 200mg/kg	208.67±	201.67±	193.67±	187.67±	181.17±	175.67±	172.33±
	1.98	2.01	1.27	1.49	1.83	1.60	1.41***

*Data are presented as mean ±SEM. The data subjected to one way ANOVA followed by Tukey's multiple comparison) at p≤ 0.05. Within the same row, # is significantly different from control group, and * is significantly different from negative control group.*

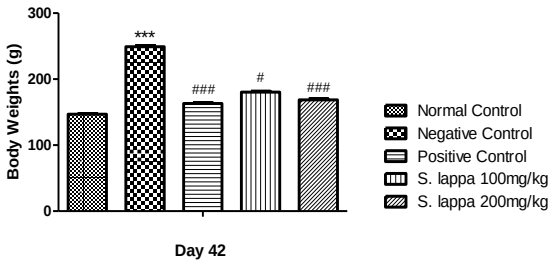

Fig2. *The body weight of experimental rats on six weeks of treatment with the ethanolic root extract of S. lappa.*

Effect of ethanolic root extract of *S. lappa* on anthropometric measures in cafeteria diet indued obese rats

Generally, changes in anthropometric parameters were observed following treatment of CD induced obese rats with ethanolic root extract of *S. lappa*. The result showed an increase in obesity index in the normal and negative control group of rats from the first week to the six weeks of treatments. On the contrary, treatment of rat with orlistat, and the two doses of the plant extract caused a consistently decrease in the obesity index from the first week to the last week of the study time.

Study of the Lee obesity index on the last week of study revealed that the CD fed untreated (Negative Control) obese rats shown an increase lee obesity index compared to other groups. However, the *S. lappa* extract and orlistat treated rats observed reduced Lee obesity index. It was found that the abdominal circumference of rats in normal control and negative control group continuously increases till end of the study. Meanwhile rats treated with the orlistat, and those treated with two extract doses indicated a decrease in abdominal circumference throughout the study. The extract treated group of rats and orlistat – treated rats showed significant ($P < 0.05$) reduction in abdominal circumference from first to the last week of the study period

Table: 4 Effect of oral administration of ethanolic extract of *S. lappa* root for six weeks on Obesity Index of cafeteria diet (CD)fed obese rats.

Treatments	Obesity Index						
mg/kg	**Day 1**	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 35**	**Day 42**
Normal Control	0.476± 0.004	0.482± 0.005	0.489± 0.005	0.494± 0.005	0.498± 0.005	0.498± 0.004	0.498± 0.004
Negative Control	0.679±	0.705±	0.719±	0.737±	0.749±	0.755±	0.758±
	0.014	0.011	0.010	0.011	0.007	0.006	0.006
Positive Control	0.672±	0.653±	0.632±	0.610±	0.587±	0.565±	0.569±
	0.004	0.007	0.007	0.006	0.006	0.006	0.004
CD + *S. lappa*100mg/kg	0.670±	0.661±	0.649±	0.646±	0.633±	0.613±	0.601±
	0.009	0.008	0.009	0.009	0.008	0.007	0.007
CD + *S. lappa* 200mg/kg	0.670±	0.648±	0.631±	0.615±	0.600±	0.587±	0.585±
	0.008	0.009	0.006	0.007	0.007	0.008	0.007

Data are presented as mean ±SEM.

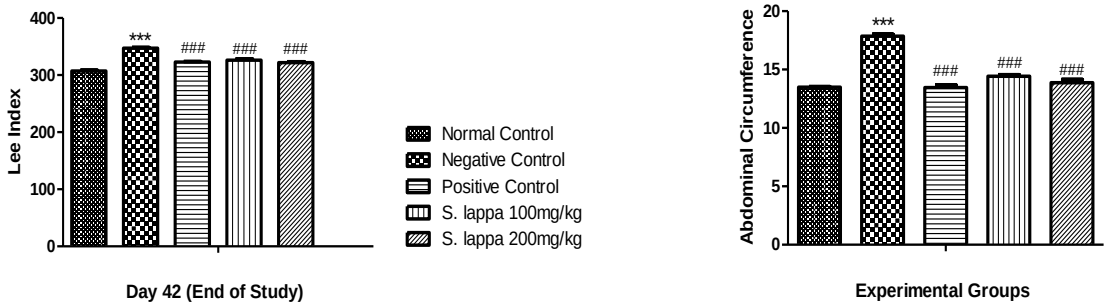

Fig3. *The Lee Index and Abdominal Circumference of experimental rats on six weeks of treatment with the ethanolic root extract of S. lappa.*

Effect of ethanolic root extract of *S. lappa* on feed intake in cafeteria diet indued obese rats

There was a significant ($P < 0.05$) increase in calorie intake per week among the cafeteria diet-fed rats as compared to the normal diet-fed rats. However, the rats treated with *S. lappa* extract doses and orlistat showed a significant ($P < 0.05$) decrease in the ratio of total feed intake to body weight of rats from the first week to the last week of study period.

Effect of ethanolic root extract of *S. lappa* on biochemical parameters

Study was observed that administration of ethanolic root extract of *S. lappa* in CD induced obese rats changed the level of serum lipid profiles and blood glucose level. Feeding of cafeteria diet caused a significant ($P < 0.05$) increase in serum levels of total-cholesterol, LDL, glucose and triglycerides as compared to normal diet fed rats.

The result showed that there was significant reduction in TG level in, positive control and the extract treated groups rats compare to negative control group rats ($P<0.05$). Finding also revealed that ethanolic root extracts of *S. lappa* shown a significant reduction of the total cholesterol level than in the negative control rats. It was statistically similar level of total cholesterol as positive control group rats ($P<0.05$). Results also indicated that the significant reduction of LDL in extract treated groups and orlistat group of as compared to negative control group of rats. ($P<0.05$) Results indicated that the level of HDL-C of rats in negative control group were no significant difference as compared to rats treated with the two extract doses and the positive control. There was no significant difference in positive control group and extract treated groups of rats as compared to negative control group. Finding indicated, treatment of ethanolic root extracts of *S. lappa* and orlistat showed the significant reduction in levels of fasting blood glucose in compare to negative control group of rats during the study. While, rats in the negative control group indicted an increased in fasting blood glucose level as compare to all other experiment groups of rats. The hypo-glycemic effect of the extracts were higher than observed in the orlistat treated group of rats at the end of study.

Table: 5 Effect of oral administration of ethanolic extract of *S. lappa* root for six weeks on Biochemical Analysis of cafeteria diet (CD)fed obese rats.

Treatments mg/kg, BW	**Biochemical Analysis**				
	TG	**TC**	**HDL-C**	**LDL-C**	**Glucose**
Normal Control	68.83±	95.33±	24.67±	51.67±	97.25±
	2.13	2.43	0.81	1.41	1.44
Negative Control	103.33±	126.17±	28.17±	100.83±	137.58±
	3.08$^{###}$	1.68$^{###}$	0.55	1.91$^{###}$	2.05$^{###}$
Positive Control	75.67±	107.83±	34.67±	68.67±	123.25±
	2.30***	1.14***	0.75	0.75***	2.15
CD + *S. lappa*100mg/kg	88.33±	117.33±	28.33±	80.67±	130.05±
	1.36**	1.83**	0.56	1.13**	1.71
CD + *S. lappa* 200mg/kg	82.33±	114.17±	29.83±	75.17±	125.29±
	0.90***	1.32***	0.47	0.78***	1.94

*Data are presented as mean ±SEM. The data subjected to one way ANOVA followed by Tukey's multiple comparison at p≤ 0.05. Within the same row, # is significantly different from control group, and * is significantly different from negative control group.*

Effect of ethanolic root extract of *S. lappa* on different adipose tissues weight and adiposity index

The weight of different fat pad such as mesenteric, retroperitoneal and epididymal, liver tissue and total fat content varied differently across the all groups of animals. The weight of mesenteric fat was significantly ($P < 0.05$) higher in CD induced obese rats than all other experimental groups. Meanwhile weight of mesenteric pad was significantly lower in the extract treated group of rats and also orlistat treated group of rats compare to negative control group. It was also shown that the weight of the retroperitoneal and subcutaneous fat pad was increase in the negative control group of rats relative to those of rats in the normal control, positive control and extract treated group ($P < 0.05$). The results revealed that rats in the negative control group showed significantly higher weight of the total fats than those of rats in the normal control, positive control and extract treated groups. The weights of total fat gradually decrease in *S. lappa* extract (200mg/kg, BW) treated group of rats as compare to negative control group of rats. Furthermore, the CD fed group of rats also induced fatty liver, with the accumulation of triglycerides when compared to the normal control group.

Table 5:-Effect of oral administration of ethanolic extract of *S. lappa* root for six weeks liver & adipose tissues weights and adiposity index of cafeteria diet (CD) fed obese rats.

S.No./Groups	Liver Weight	Adipose Tissue Weights			Total Adipose Tissue	Adiposity Index
		Epididymal	Mesenteric	Retroperitoneal		
Normal Control	11.04±	3.12±	2.63±	1.80±	7.55±	5.14±
	0.26	0.11	0.12	0.09	0.11	0.04
Negative Control	21.54±	6.50±	6.07±	5.21±	17.78±	7.14±
	$0.44^{###}$	0.10	0.08	0.10	$0.19^{###}$	$0.10^{##}$
Positive Control	12.38±	3.56±	3.07±	2.50±	9.13±	5.60±
	0.39^{**}	0.10	0.08	0.15	0.16^{***}	0.11^{***}
CD + *S. lappa*100mg/kg	16.40±	4.07±	3.98±	3.18±	11.23±	6.22±
	0.65^{***}	0.21	0.18	0.10	0.35^{**}	0.16^{***}
CD + *S. lappa* 200mg/kg	14.54±	3.90±	3.52±	2.67±	10.09±	5.86±
	0.18^{***}	0.04	0.08	0.10	0.12^{***}	0.11^{***}

*Data are presented as mean ±SEM. The data subjected to one way ANOVA followed by Tukey's multiple comparison at p≤ 0.05. Within the same row, # is significantly different from control group, and * is significantly different from negative control group. Adiposity index was expressed as Total Adipose Tissue × 100/ body weight (g)*

DISCUSSION

The genetic, physiological, epigenetic, environmental, and nutritional variables that cause obesity in vulnerable people or animals have been thoroughly explored as part of the pathogenesis of obesity[9, 10]. The present phytochemical investigation of this plant showed that ethanolic extract of root is enriched with important phytochemical such as flavonoids, phenols terpenoids, saponins and tannins. The Ethanolic extract of *S. lappa* root were also found total flavonoids and total phenolic content. Current scientific evidence on the possible effects of flavonoids in counteracting obesity and related co-morbidities through a decrease in oxidative stress and related inflammatory conditions[32, 33]. Earlier researcher also reported the significance presence of these phytochemical such as found flavonoids, terpenoids, saponin, alkaloids, cardiac glycosides, carbohydrate, and different polyphenols in root extracts of *S. lappa*[14].

The study results reveal that the extracts of *S. lappa* shown the greatest pancreatic lipase and α-glucosidase inhibition efficiency. Interestingly, Ethanolic extract of *S. lappa* exhibited valuable degrees of antioxidant activity by using DPPH free radical scavenging assay. Besides flavonoids influence fat digestion through inhibition of small intestine micelle formation and the inhibition of alpha- glucosidase activity leading to a decrease in triacylglycerol absorption. Maintaining the balance between α-amylase and α-glucosidase inhibitors would be useful to decrease the gastrointestinal adverse effects related to undigested starch reaching the colon. Strong positive correlation between phenolic and flavonoid compounds and that of free radical scavenging/antioxidant activity and enzyme (alpha-amylase, alpha-glucosidase and pancreatic lipase) inhibition activity were observed, which suggested that these compounds were mainly responsible to the antioxidant, antidiabetic and anti-obesity potential observed. Therefore, the effect of flavonoids/terpenoids/ saponins/tannin

rich ethanolic extract of roots of *S. lappa* on preventing obesity was further explored in this study using various animal models.

The ethanolic root bark extract of *S. lappa* did not produce any toxic symptoms of mortality up to the dose level of 2000 mg/kg body weight in rats, and hence the drugs were considered safe for further pharmacological screening, the 100mg/kg/day and 200mg/kg/day were taken as dose for the evaluation of anti-obesity activity. The present study evaluated the effect of the ethanolic root extracts of *S. lappa* in cafeteria diet induced obese rats. Finding shown that rats fed with a variety of highly palatable, energy rich, high carbohydrate cafeteria foods produced obesity-like conditions, significant increase in body weights and serum cholesterol, triglycerides, glucose, and a decrease in serum HDL-cholesterol and various adipose tissue weight in the cafeteria fed group relative to the normal control group throughout the study period. Cafeteria diets have been previously reported to increase energy intake and cause obesity in humans as well as animals[17, 20, 21]. Further the composition and variety of cafeteria foods also exert synergistic effects on the development of obesity[18, 19].

Oral treatment of *S. lappa* root extract caused significant reduction in body weight as compare to negative control group rats ($P < 0.05$). This similar effect was also observed that with orlistat, positive control group of rats. Orlistat, an approved anti-obese drug is clinically reported to prevent obesity and hyperlipidemia through the increment of fat excretion into the feces and inhibiting pancreatic lipase resulting in reduced dietary fat absorption. This effect to reduce the bodyweight of the experimental rats by the extract might be due to singly, additive and/or synergistic effect of contained phytochemicals. Emerging studies have described the promising role of flavonoids in treating obesity and diabetes as well as their associated metabolic diseases[11]. Some of the phytochemical constituents, such as saponins, flavonoids, and some triterpenoids, have been reported for their anti-obesity effect in various plants[32, 34]. The anti-obesity and anti-diabetic potential associated with flavonoids are very large given their regulatory effects on blood sugar transporters by increasing insulin secretion, reducing apoptosis, promoting pancreatic β-cell proliferation, and reducing insulin resistance, inflammation, and oxidative stress in the muscle[11].In fact, many polyphenols, including flavones, flavanols, tannins and chalcones, have shown an inhibitory activity of pancreatic lipase [35]. Finding suggested the variety of plants likes *Panax japonicus, Platy-codi radix, Salacia reticulata, Nelumbo nucifera* possess pancreatic lipase inhibitory effects due to phytochemicals found include mainly saponins, polyphenols, flavonoids and caffeine[36,37]. Researcher revealed that ethanolic seeds extract of papaya flavonoids compounds likes epicatechin, catechin or epigallocatechin-3-gallate has predicted pancreatic lipase inhibitors like orlistat[38].

Saponins are known bioactive substances that can reduce the uptake of cholesterol and glucose at the gut through intra-lumenal physiochemical interaction. It has been reported that the saponins in *Panax ginseng, Platycodi radix*, and *Panax japonicus* rhizomes, all belonging to the family of the triterpenoid family of saponins, showed strong inhibitory effects on the pancreatic lipase in vitro and suppressed the increase in body weight induced by a high-fat diet in vivo[39, 40]. Saponins from ginseng roots suppress the expected increase in body weight and plasma triacylglycerols in mice following a high-fat diet, which was probably mediated by inhibiting PL with an IC50 value of 500 μg/mL[41].Researcher observed that Saponin-rich extracts and their hydrolysates from fenugreek and quinoa and saponin and sapogenin standards, were assessed on the inhibition of pancreatic lipase and interference on the bioaccessibility of cholesterol by *In vitro* digestion models[42].

The result revealed that there was a cumulative increase in food intake in obese untreated group of rats relative to other experimental groups. This result suggests that the body weight reducing effect of *S. lappa* extract in cafeteria diet-fed rats may be produced due to its hypophagic property. Previous studies have reported that cafeteria diet induce hyperphagia in rats due to down regulation of striatal D2 receptor expression is a notable neuroadaptive response to over consumption of palatable food which results seen in overweight individuals[17, 18]. The observed decrease in the quantity of feed in take upon treatment with the plant extract could be attributed to its potential to increased satiety signals that mediate reduction of food intake, decrease body weight and increases the energy expenditure[19, 20].The presence of tannins in *S. lappa* extract contribution to reduction in feed intake by decreasing palatability. Previous studies have reported that eating behavior is modulated by brain reward system through the mechanism that involve the

homeostatic need to feed as well as the hedonic and cognitive value of ingestion[16].

Treatment of *S. lappa* root extract reduced the Lee index, Obesity index and Abdominal circumference throughout the study period. The observed reduction in these values might be due to the presence of terpenoids which promotes weight reduction through suppressed de novo lipogenesis, increase lipid oxidation and reduced food intake. The result also revealed that the obese untreated-rats fed with cafeteria diet shown continuous increase in obesity index and abdominal circumference from first to six weeks of study. Meanwhile, there was 2-3 times increase in liver and adipose tissue weight in cafeteria diet induced negative control group of rats compare to remaining group of rats. Finding suggested that exposure of rats to calorically dense diets elevated fat accumulation in the abdominal region and also decrease the resting metabolic rate or diet induced thermogenesis. Therefore, Diet induced obesity led to an increase in number of adipocytes and their size[21]. Significant increase in serum lipids profile, such as total cholesterol, LDL-C, and triglycerides have been shown to be significant predictors for metabolic syndrome. The elevation of serum TGs in untreated group of rats fed with cafeteria diets is indicative of increased *de-novo* lipid biosynthesis[20, 40]. Our finding suggested that administration of *S. lappa* root extracts decreased the levels of TC, TG, LDL and VLDL but increased HDL compare to negative control group of rats. The decrease in the body adiposity index, epididymal, mesenteric and retroperitoneal adipose weight in ethanolic extract of *S. lappa* treated group of rats may be due to inhibitory effect in the formation of new adipocytes from preadipocyte cell or decreased adipocyte size due to fat storage (adipocyte hypertrophy).The reduction in body weight, liver weight and the liver -TG content of *S. lappa* treated group of rats shown protective action against obesity and related metabolic complication like CVD, dyslipidemia and insulin resistance etc.

Results also revealed that significant elevation in blood glucose level in cafeteria diet induced untreated rats relative to extract treated group of rats. Increased level of circulation blood glucose in characteristics of hyperglycemia as a result of an insulin resistance due to reduced number of insulin receptors, impaired insulin receptors binding and disruption in post receptor insulin signaling transduction. Various polyphenol and flavonoids extracts are able to decrease the blood levels of glucose, triglycerides and LDL cholesterol, increase energy expenditure and fat oxidation, and reduce body weight and adiposity[26, 31,32]. In this study, the polyphenols, flavonoids, triterpenoids and saponins found to be present in the root extracts *S. lappa* might be responsible for the management of the obesity and other metabolic abnormalities. The phytochemicals might be provided synergistic effect for regulation of various pathways including reduction in lipid absorption, maintain energy intake and expenditures, inhibition of lipase activity, and consequently, the reduction of body weight in cafeteria diet-induced obese rats.

CONCLUSION

In summary, Ethanolic extract of *S. lappa* root might exert its anti-obesity action through the inhibition of intestinal absorption of dietary fat and triglyceride by lipase enzymes, its hypophagic activity, and its hypolipidemic activity may be due to its great free radical scavenging activity and active phytoconstituents. Thus, it is suggestive that the *S. lappa* root extract used in this present study, which contains flavonoids, triterpenoids, tannins and saponins reduced the fat accumulation in the cafeteria diet fed obese rats by inhibiting the activity of pancreatic lipase. Further studies are needed to identify and purify the specific phytoconstituents of *S. lappa* root which is responsible for its anti-obese activity,to determine the molecular mechanisms likes control the gene expression of adipocyte differentiation-related transcription factors, lipogenic and lipolytic enzymes involved in glucose and lipid metabolism in obesity and diabetes would provide insight into the field of drug development, and future discoveries are expected to yield therapeutic benefits.

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