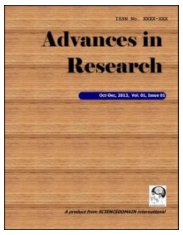

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Anti-Obesity Potential of Gallic Acid from *Labisia pumila*, through Augmentation of Adipokines in High Fat Diet Induced Obesity in C57BL/6 Mice

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The incidence of obesity has increased at an alarming rate in recent years, becoming a worldwide health problem. Anti-obesity drugs available have hazardous side-effects, thus, a wide variety of natural materials are being explored for their anti-obesity potential. This study was undertaken to investigate the activity of Gallic acid (GA), a compound isolated from aqueous leaf extract from *Labisia pumila* (LPPM/A003) on its potential to prevent obesity.
Place and Duration of Study: The study was carried out at Department of Pharmacology, Indian Institute of Integrative Medicine, Council of Scientific and industrial research, Jammu (J and K) for duration of 12 months.
Methodology: All the test materials were initially screened for *In-vitro* adipocyte differentiation. The active sample was selected for *In-vivo* anti-obesity effect in high fat diet induced obesity in C57BL/6 mice and the biochemical and molecular parameters were measured.
Results: In *In-vitro* screening, both LPPM/A003 and GA had inhibitory effect on fat droplet formation and triglyceride accumulation. The concentration at which GA showed 50% inhibition was 19.86µg/ml. The *In-vivo* studies in obese mice decreased the weight in GA

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treated animals. Excessive secretion of leptin in case of obesity leads to disrupted functions of hypothalamus and GA showed promise by inhibiting Leptin. Obesity is considered as a state of low-grade inflammation and inflammation is regarded as cause or consequence of obesity. It also inhibited the increased expression of TNF- α and IL-6 in serum of treated animals. GA inhibited the increase in serum levels of Triglyceride, LDL and VLDL. HDL levels were elevated at all the dose levels with significant increase at 8mg/kg dose.

Conclusion: Gallic acid is an effective compound capable of modulating diet induced weight gain in obese mice. Research in this field paves the way to discover new treatments for obesity.

Keywords: Gallic acid; high fat diet induced obesity; adipokines; leptin; Labisia pumila.

ABBREVIATIONS

GA: Gallic Acid; LPPM/A003: Aqueous Extract of leaves of Labisia pumila; PG: Pyrogallol C57BL/6: C57 black 6 inbred strain of laboratory mice; TNF-α: Tumour necrosis factor-alpha; IL-6: Interleukin-6; ORO: Oil-Red-O; IC_{50}: Concentration at which 50% inhibition; HDL: High Density Lipoprotein; LDL: Low density lipoprotein; VLDL: Very Low Density Lipoprotein.

1. INTRODUCTION

Good nutrition, physical activity, and a healthy body weight are essential parts of a person's overall health and well-being. Our current obesogenic environment promotes increased food intake, non-healthful foods and physical inactivity, so weight gain and disease are really natural and expected consequences of this obesogenic environment. Natural product (nutraceutical) interventions are currently being investigated on a large-scale basis as potential treatments for obesity and weight management. Ethno botanical claims indicate that progress has been made concerning our knowledge of bioactive components in traditional plants and their links to obesity. With a global increase in the prevalence of obesity, nutraceuticals plays a key role in its prevention and treatment.

Obesity is an excess of body fat that frequently results in a significant impairment of health. It is the most prevalent nutritional disorder and is described as a global epidemic. Obesity results when the size or number of fat cells in a person's body increases resulting as a possible gateway for various chronic physical and psychological problems. It increases the likelihood of various diseases that include heart disease, type 2 diabetes, obstructive sleep apnea, certain types of cancer, and osteoarthritis [1]. It is most commonly caused by a combination of excessive food energy intake, lack of physical activity and genetic susceptibility, although, a few cases are caused primarily by endocrine disorders.

Obesity is characterized by increased adipose tissue mass that results from both increased fat - cell number (hyperplasia) and increased fat-cell size (hypertrophy) [2]. The number of adipocytes present in an organism is determined to a large degree by the adipocyte differentiation process, which generates mature adipocytes from fibroblast-like preadipocytes. A fat cell develops as internally produced lipid droplets coalesce into a single large mass. Eventually, cellulite results due to enhanced adipogenesis and accumulation of adipocytes under the skin.

Adipose tissue, once thought to function primarily as a passive depot for the storage of excess lipid, is now understood to play a much more active role in metabolic regulation, secreting a variety of metabolic hormones [3]. Adipose tissue is currently known to secrete a large number of proteins termed adipokines that act in an autocrine, paracrine, or endocrine fashion to control various metabolic functions. Leptin is an important adipocyte hormone that, influences food intake through a direct effect on the hypothalamus. In obese individuals, leptin concentrations are already high because of the increased amount of leptin-secreting adipose tissue [4]. Eexcessive secretion of leptin as in case of obesity or experimental models of induced obesity leads to disrupted functions of hypothalamic centres that an obese subject tends to go on a over feeding mode. Hence, it becomes imperative to bring about effective reduction of the over expressed levels of leptin in obesity.

Several pro-inflammatory factors are produced in adipose tissue with increasing obesity. Compared with that of lean individuals, adipose tissue in obese persons shows higher expression of pro-inflammatory proteins, including TNF-α, interleukin 6 (IL-6), monocyte chemotactic protein 1, inducible nitric oxide synthase etc. [5]. Macrophage numbers in adipose tissue also increase with obesity [6], where they apparently function to scavenge adipocytes, which increase dramatically with obesity [7]. Macrophages are responsible for most of the cytokine production in obese adipose tissue. Although triglycerides are essential for normal physiology, excess triglyceride accumulation occurs in obesity and is also associated with insulin resistance.

Labisia pumila or more commonly known as Kacip Fatimah has been used widely in South East Asian communities for a variety of illnesses and in food supplements. This plant has been widely used by Malaysian women for generations for its postpartum rejuvenating properties and toning of abdominal muscles [8]. In literature the weight regulating activity of Labisia aqueous extract is reported in ovariectomized rats [9]. We wanted to look for the weight management effect in high fat diet induced obese mice. During the course of study we found, aqueous extract of Labisia pumila (LPPM/A003) to have anti-obesity potential and further study was undertaken to identify the compound present in LPPM/A003 responsible for anti-obesity potential.

LPPM/A003 and two compound i.e., Gallic acid (GA) and Pyrogallol (PG) isolated from this extract were screened through in-vitro experimentation for anti-obesity potential where GA was found to have most significant effect and was then taken up for detailed *In vivo* studies in high fat diet induced obese mice to look into the various different aspects and targets related to obesity.

2. MATERIALS

2.1 Plant Material

Plant material was collected from Kawasan Tasik Banding, Grik, Perak Malaysia on November 2007and authenticated by Forest Research Institute of Malaysia. A voucher sample (Accession no. 21648) is retained and deposited at the Herbarium of this institute.

2.1.1 Preparation of LPPM/A003 extract

Dry Powdered leaves of *L. pumila* (500g) were soaked in distilled water (4.0 litre) and the contents were stirred at 70ºC for 3hrs. The contents were then filtered and the marc taken in

fresh 4.0 litres of water and the process was repeated twice (total extractions =three) keeping the temperature and time parameters constant. The total filtrate ~ 10 litres was concentrated at 50ºC and reduced to two litres volume and then freeze dried to get 47.5g light brownish free flowing powder labeled as LPPM/A003. The drug-extract ratio (DER) was 100:9.5 (w/w).

2.1.2 HPLC of LPPM/A003

The HPLC analysis was done on Bruker Daltonics Esquire 3000 (LC) Agilent 1100 series instrument using RP-18 (Merck, 5µm, 4x250mm) column, temp 30°C at wavelength 270nm with flow rate of 0.5ml/min. Mobile phase was used in gradient as solvent A (water containing 0.1% formic acid) and solvent B (acetonitrile),

Time (mins):	0	5	50	55	60	65
Solvent B (%):	5	5	55	55	5	5

The aqueous extract was standardized on the basis of marker compounds Gallic acid (GA) and Pyrogallol (PG) isolated and well characterized from the leaf extract of plant. On quantification, the concentration of Gallic acid and Pyrogallol was found to be 1.98% and 0.0015% respectively. However, the extract was found to be rich in polyphenolic compounds. HPLC of LPPM/A003 is shown in Fig. 1. The HPLC graph of standard gallic acid (Rt=4.60min) and pyrogallol (Rt=6.07 min) at wavelength 270nm and 254nm is also shown in the Fig. 1 [10].

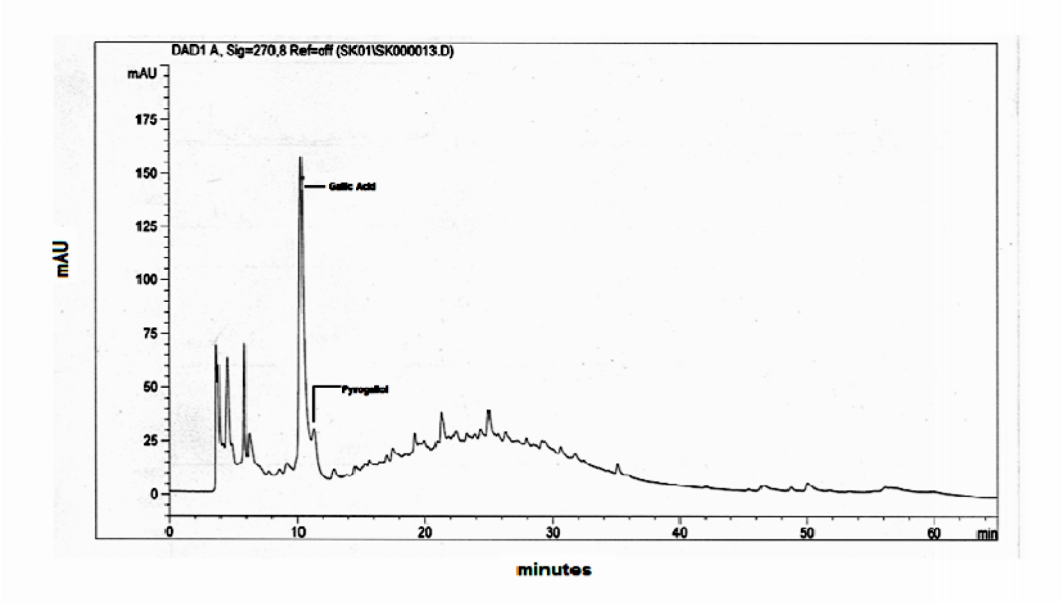

Fig. 1. HPLC graph of LPPM/A003 with separation of gallic acid (Rt=4.64 min) and pyrogallol (Rt=6.31) at two different wavelengths

2.2 Animals

C57BL/6 male mice aged 3 weeks old were taken and were fed with High fat diet (60% Kcal % Fat) upto 19 weeks; these were then taken for the study to assess anti-obesity potential of the test material. Animals were housed under standard laboratory conditions with adequate

fresh air supply (Air changes 12-15 per hour), room temperature 21.0-23.4ºC, relative humidity 55-61%, with 12 hours light /dark cycle. All experimental procedures used in present study were in accordance with institutional guidelines for animal research (CPCSEA, 2003). The study protocols were approved by the Institutional Animal Use and Care Committee of Indian Institute of Integrative Medicine, Jammu.

3. EXPERIMENTAL PROCEDURES

3.1 Adipogenesis Inhibitory Assay in 3T3-L1 Mouse Adipocytes (*In vitro*)

A terminal differentiation of adipocytes is accompanied by the accumulation of great amounts of lipids in large cytoplasmic vesicles. A common assay to measure adipocyte differentiation in cell culture is with the dye Oil Red-O (ORO). ORO is a lipid-soluble bright red dye which is a reliable indicator of adipocyte differentiation [11]. Oil Red O (Solvent Red 27, Sudan Red 5B, C.I. 26125, and C26H24N4O) is a lysochrome (fat-soluble dye) diazo dye used for staining of neutral triglycerides and lipids. 3T3-L1 cells approximately $60X10^4$ cells were seeded for 48-72hrs to get 70-80% confluence. After 48 hrs 200µl of AIM (Adipogenesis induction medium) freshly prepared was added. 72hrs later 200µl APM (Adipogenesis progression medium) with the test samples in different concentrations was added to the wells. The cells were incubated for 48hrs in a humidified atmosphere (37ºC) of 5% CO_2 and 95% air. [12]. Cells were fixed by adding 100µl of 10% formalin and ORO staining was done. OD was read at 492 nm in microplate reader and the percentage inhibition was calculated. The results were expressed as IC_{50} values using Graphpad prism software and the percentage inhibition of adipogenesis in test drug treated samples were calculated [13].

3.2 Induction of Obesity

Ten animals were allotted to Group 1 and served as Normal Diet Control Group and fed with Diet (10% Kcal % Fat) for 19 weeks. Forty animals were allotted to Group 2 and fed with High Fat Diet (60% Kcal % Fat) for 19 weeks to induce obesity [14]. Obesity induction was confirmed based on the comparison of High Fat Diet animals body weights with Normal Diet animals. The animals which were found non obese were eliminated from the study.

To assess the therapeutic effect, GA was administered to obese mice daily for period of 28 days at 2, 4 and 8mg/kg dose levels. Weekly twice body weights and feed intake was recorded.

3.3 Study Design

Eight animals from Normal Diet group selected for the main study were assigned as Control Group. Thirty two obese animals from High Fat Diet groups were selected and allotted to four groups (8 animals per group) as High Fat Diet Control Group, Test drug groups included administration of GA at dose levels of 2, 4 and 8mg/kg respectively (Table 1).

Table 1. Study design

Experimental Groups	Dose (mg/kg)	No. of Animals
Normal Control	0	8
High Fat Control	0	8
High Fat Diet + GA-2	2	8
High Fat Diet + GA-4	4	8
High Fat Diet + GA-8	8	8

3.4 Mean Body Weight

Individual body weight was recorded once on the day of commencement of treatment and on days 4, 8, 11, 15, 18, 22, 25 and 28th of the experimental period.

3.5 Feed Consumption

The food consumption was measured on the day of body weight measurement during treatment period. The cage wise average food intake (g/mouse/day) was calculated.

3.6 Blood Collection and Biomarker Estimation

On Day 29 of experiment, blood was collected from all animals in the groups through retro orbital plexus. Serum was separated from whole blood by centrifugation and processed for lipid profile analysis and molecular target estimation.

3.7 Estimation of Leptin

Estimation of Leptin was done by ELISA kits from R and D systems according to manufacturer's information in the serum samples of the experimental animals.

3.8 Serum Biochemistry

The serum lipid profile was analyzed using the "EM-360 Fully automated clinical chemistry analyzer" (Transasia Bio-Medicals Ltd., India). The total cholesterol, Triglycerides, HDL (High density lipoprotein), LDL (Low density lipoprotein) and VLDL (Very low density lipoprotein) were measured in mg/dL.

3.9 Estimation of Pro-Inflammatory Markers

Serum was collected from the experimental animals and estimation of pro-inflammatory markers viz. TNF-alpha and IL-6 was estimated by ELISA kits from R and D systems according to manufacturer's information.

3.10 Acute Safety Study

The acute toxicity study was performed in compliance with Schedule ''Y" [15] and Good Laboratory Practices requirements of Govt. of India. In the acute studies, male and female Swiss albino mice were used. In experiment designed to determine oral LD_{50} of GA in swiss albino mice (6/sex/group; 10–11 weeks of age; body weight range: males 28-35g, females

25–33g) were administered a single oral (gavage) dose of GA at 50, 500, 1000 and 2000 mg/kg body weight and animals were observed for 14 days for any signs of morbidity or mortality. Similar set of experimentation was performed in wistar rats (6/sex/group; 10–11 weeks of age; body weight range: males 178–260g, females 147–200g).

3.11 Statistical Evaluation

The mean and standard error (S.E.) of the mean for each group was calculated and the results were expressed as percent inhibition compared with the control group. The significance was determined statistically by applying Student's t-test.

4. RESULTS

4.1 Adipogenesis Inhibitory Assay in 3T3-L1 Mouse adipocytes *(In vitro*)

A broad concentration range of 3.12, 6.25, 12.5, 25, 50 and 100 µg/ml was tested. The concentration at which LPPM/A003 showed 50% inhibition (IC_{50}) was 94.51µg/ml and that of GA was 19.86µg/ml, however**,** IC_{50} value was not obtained for PG upto the highest workable concentration (Table 2). Lower the IC_{50} (µg/ml) value, better is the efficacy. Fig. 2 shows ORO treated control cells and inhibition of adipogenesis in sample treated cells. During adipocytes differentiation GA had significant inhibitory effect on fat droplet formation and triglyceride accumulation

Table 2. Inhibition of adipocyte differentiation by different concentrations of test samples in 3T3 L1 adipocyte cells

Sample	Percent inhibition at graded concentration (µg/ml) →				←		IC_{50} µg/ml
	3.12	**6.25**	**12.5**	**25**	**50**	**100**	
LPPM/A003 (Aqueous extract)	-NIL-	6.24%	11.7%	28.15%	41.17%	52.19%	94.51
PYROGALLOL (PG)	-NIL-	8.91%	9. 58%	24.21%	26.66%	31.44%	-
GALLIC ACID (GA)	14.15%	21.67%	37.76%	53.55%	70.12%	76.45%	19.86

The results are expressed as IC_{50} values using Graphpad prism software. This is the concentration at which 50% inhibition of adipogenesis takes place. Lower the IC_{50} (µg/ml) value, better is the efficacy

4.2 Mean Body Weight

The data obtained from Adipogenesis inhibitory assay in 3T3-L1 mouse adipocytes showed GA to have the most significant activity when compared to PG and LPPM/A003.

Detailed study was taken up to look into the anti-obesity effect of GA (*In vivo*) and ascertain the anti obesity activity to this marker compound.

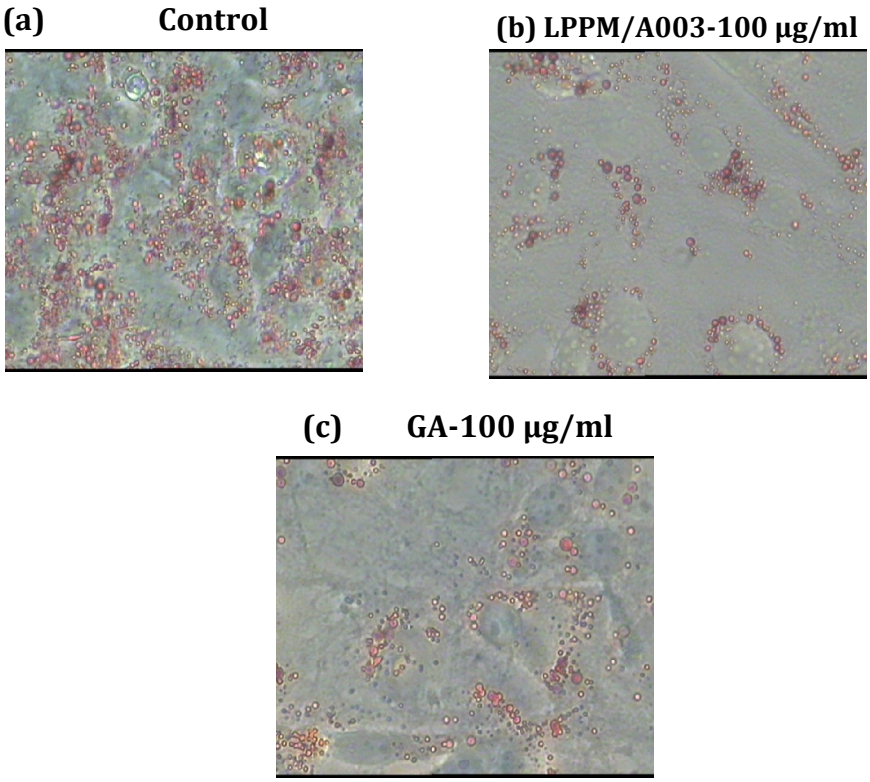

Fig. 2. ORO stained mouse 3T3L1 cells (a) Control cells (b) Cells treated with LPPM/A003-100µg/ml (c) Cells treated with GA-100µg/ml

There was a significant increase in body weight of high fat diet fed group animals when compared to normal control group (Table 3). Mice on high fat diet when treated with GA (2, 4 and 8mg/kg, p.o.) showed a significant decrease in body weight when compared to body weights of mice in high fat diet control group animals (Fig. 3). Broad range of doses was taken for the study (data not shown) but the effect was prominent at the dose level of 2, 4 and 8mg/kg p. o.

4.3 Effect on Feed Consumption

The food consumption was measured on the day of body weight measurement during treatment period. There were no significant changes in average food intake (g/mouse/day) observed at tested dose levels of test item for 28 days in therapeutic dose when compared to High fat diet control (Table 4).

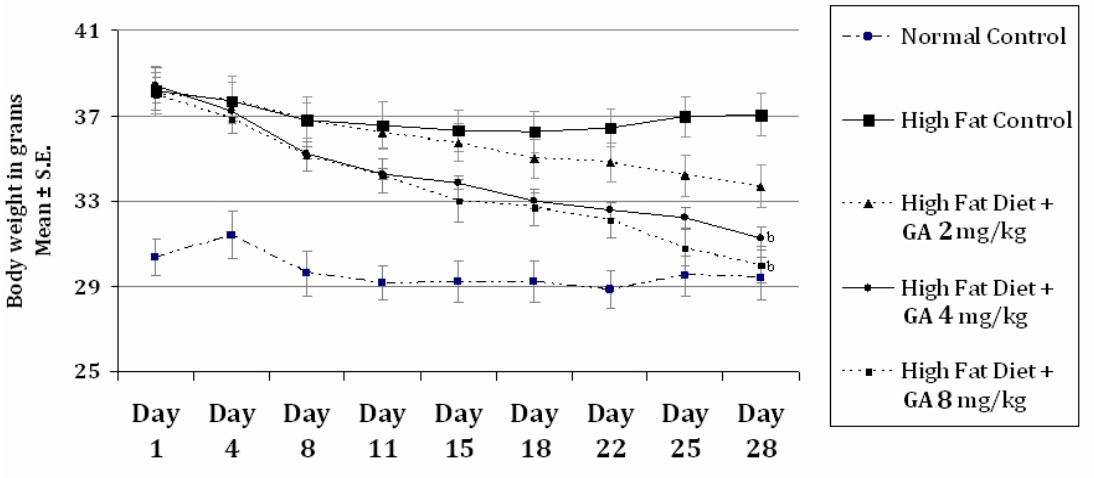

Fig. 3. The figure represents Mean body weight in grams of normal control group, high fat diet control group and GA treated test groups

b = HFD Vs HFD + Test item treated ($p < 0.05$); GA: Gallic acid at 2, 4 and 8mg/kg p. o. n = 8 per group

4.4 Inhibition of Leptin Expression in GA Administered Obese Rats

A decrease in levels of leptin concentration in groups treated with graded doses of GA was observed when compared to High fat diet fed group (Fig. 4). GA was found effective in reducing of the over expressed levels of leptin in obese experimental animals.

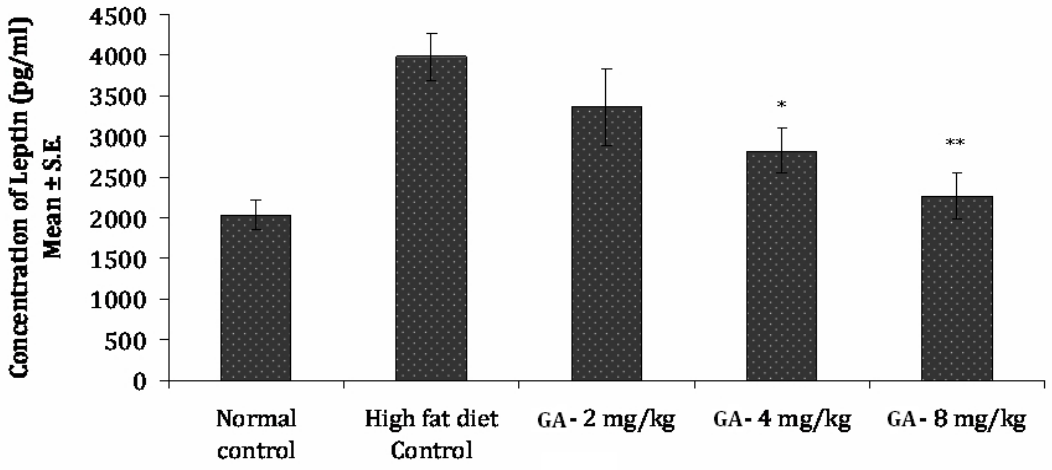

Fig. 4. Figure represents concentration of Leptin produced in normal control group, high fat diet control group and GA treated test groups

**= $p<0.05$; ** = $p<0.01$; GA: Gallic acid at 2, 4 and 8 mg/kg p. o. n=8 per group*

4.5 Effect of Oral Administration of GA on Lipid Profile in Test Groups

Obesity or high-fat diet is associated with insulin resistance which in turn is associated with an increase in plasma concentrations of cholesterol, triglycerides, or both and is caused by defects in the metabolism of the lipoprotein classes, very-low-density lipoprotein (VLDL), low density lipoprotein (LDL), and high-density lipoprotein (HDL). Chronic obesity resulted in a significant increase in serum cholesterol and triglyceride concentrations in high fat diet fed animals. In GA (2, 4 and 8mg/kg) treated groups, serum cholesterol levels were reduced at 8 mg/kg in comparison with high fat diet (HFD) control. Compared with normal control-group animals, obese-group animals had higher concentrations of cholesterol in total serum (192.63 mg/dl) and in VLDL (30.35mg/dl) and LDL (86.45 mg/dl) and decrease levels of HDL (28.5 mg/dl). Significant reduction was observed in serum Triglyceride and VLDL levels at tested dose levels. HDL levels were slightly elevated at 4 and 8mg/kg and significant increase was observed at 8mg/kg dose and also the elevated levels of LDL was decreased by administration of GA at graded doses (Table 5).

4.6 Effect of Graded Doses of GA on Pro-Inflammatory Cytokines Associated With Obesity

The serum from the treatment groups was collected and subjected to quantikine estimation of TNF-α and IL-6 by R and D Systems ELISA kit. GA significantly decreased the TNF-α level in a dose dependent manner showing significant inhibition at higher dose levels of 8 mg / kg per oral. Predictably we noted a higher percentage expression of 128.40±16.79GA/ml of TNF-α in high fat diet control group when compared to 25.52±1.83% expression in naïve control. Maximum suppression was observed at 8mg/kg p.o. dose (Fig. 5). Up-regulation of IL-6 is considered to be involved in the pathological process of obesity. IL-6 increases lipolysis and fat oxidation in humans [16]. This study clearly show that high fat diet induced obese mice given GA had considerably reduced amounts of IL-6 protein expressed by the adipocytes in the peripheral blood. The expression of IL-6 in GA 4 and 8mg/kg dose was 15.32±2.14 and 14.77±2.76 respectively (Fig. 6). This finding was in sharp contrast to high fat diet control group mice where higher amounts of IL-6 (25.5±1.89) were detected in all of the obese animals analyzed. Thus oral administration of GA at dose levels of 2, 4 and 8 mg/kg appears to inhibit the production of IL-6 in the serum samples of experimental mice.

4.7 Acute Safety Study

Aqueous extract of *Labisia pumila* is safe [17]. The 14-day observation period during the acute oral toxicity study and body weight measurements did not reveal any toxic effects in either species. In the acute toxicity studies, oral LD_{50} of GA in Swiss albino mice was greater than 1000mg/kg body weight, LD_{50} dose obtained from series of experiments was 1580mg/kg in swiss albino mice. GA did not show any change in gross general behaviour of these test animals at the lower doses.

Table 3. Effect on mean body weight by oral administration of GA at graded doses

Treatment groups	Body weights (g)								
	Day 1	Day 4	Day 8	Day 11	Day 15	Day 18	Day 22	Day 25	Day 28
Normal Control	30.35±1.17	31.4±1.09	29.62±1.05	29.14±1.09	29.2±0.97	29.22±0.97	28.84± 0.9	29.5±0.93	29.37±0.99
High Fat Control	38.17±0.96[a]	37.72±0.85[a]	36.8±0.82[a]	36.54±0.77[a]	36.31±0.86[a]	36.23 ±0.91[a]	36.43±0.92[a]	36.94±0.98[a]	37.06±0.98[a]
High Fat Diet + GA 2 mg/kg	38.04±0.86	37.73±0.48	36.77±0.27	36.19±0.24	35.73±0.32	35.0±0.39	34.80±0.41	34.18±0.49[a]	33.69±0.55
High Fat Diet + GA 4 mg/kg	38.42±0.73	37.19±0.65	35.24±0.69	34.26±0.79	33.87±0.98	33.00±0.83	32.59±0.82[b]	32.21±0.88[b]	31.27±0.85[b]
High Fat Diet + GA 8 mg/kg	38.02±1.26	36.84±1.17	35.09±1.4	34.18±1.47	33.02±1.62	32.70±1.79	32.10±1.84[b]	30.80±1.82[b]	30.02±1.83[b]

Values are expressed as Mean ± SEM; n= 8; a = Normal Vs HFD (p<0.05); b = HFD Vs HFD + Test item treated (p<0.05)

Table 4. Table represents group mean feed consumption

Treatment groups	FEED consumption (g)							
	Day 1-4	Day 4-8	Day 8-11	Day 11-15	Day 15-18	Day 18-22	Day 22-25	Day 25-28
Normal Control	5.05±0.34	3.61±0.5	4.59±0.72	7.29±0.4	5.48±0.21	5.48±0.21	5.26 ±0.58	5.49±0.35
High Fat Control	2.33±1.03	5.36 ±0.84	5.56±0.49	8.56±0.56	5.08±0.38	6.11±0.41	5.61±0.23	5.65±0.16
High Fat Diet + GA 2mg/kg	3.93±0.8	7.89±1.08	6.15±0.48	8.79±0.38	4.59±0.24	6.65±0.39	5.91±0.19	5.78±0.16
High Fat Diet + GA 4mg/kg	1.94±0.58	6.8 ±0.86	6.68±0.36	8.81±0.32	4.53±0.28	7.46±0.72	5.1±0.5	5.24±0.35
High Fat Diet + GA 8mg/kg	2.06±0.39	7.06±0.8	5.94±0.2	11.49±0.45	4.38±0.22	6.58±0.22	4.95±0.36	4.98±0.17

Values are expressed as Mean ± SEM; GA: Gallic acid at 2,4 and 8 mg/kg p.o.; n = 8 per group

Table 5. Table represents serum lipid profile of the experimental groups

Treatment groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Normal Control	138.13±21.24	63.63±8.07	27.86±1.6	72.31±7.37	12.73±1.61
High Fat Control	192.63±11.07[a]	151.75±28.9[a]	28.5 ±1.72	86.45±11.08	30.35±5.78[a]
High Fat Diet + GA 2 mg/kg	160.75±25.44	92.13±6.73[b]	29.34±1.95	78.1±13.21	18.43±1.35[b]
High Fat Diet + GA 4 mg/kg	152.13±15.08	84.63±12.85[b]	30.31±1.11	72.88±10.76	16.93±2.57[b]
High Fat Diet + GA 8 mg/kg	150.13±14.21[b]	56.63±10.84[b]	31.94±1.95[b]	64.4±10.24[b]	11.33±2.17[b]

VLDL= Very-low-density lipoprotein cholesterol. HDL = High-density lipoprotein. LDL = Low-density lipoprotein; Values are expressed as Mean ± SEM; n= 8 a = Normal Vs HFD (p<0.05) b = HFD Vs HFD + Test item treated (p<0.05)

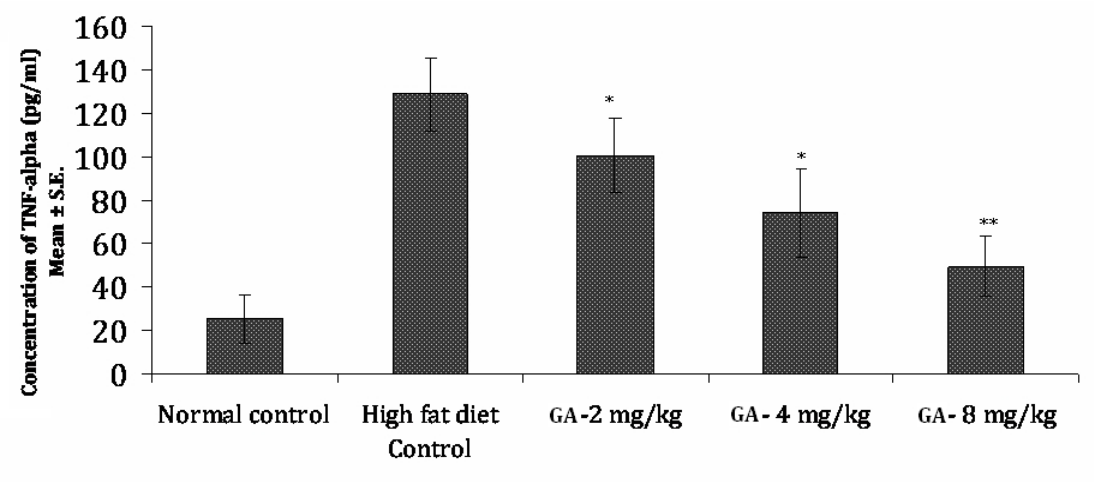

Fig. 5. Expression of TNF-α in serum from high fat diet induced obese C57BL/6 mice treated with different concentration of GA

**= p<0.05; ** = p<0.01; GA: Gallic acid at 2, 4 and 8mg/kg p.o; n = 8 per group*

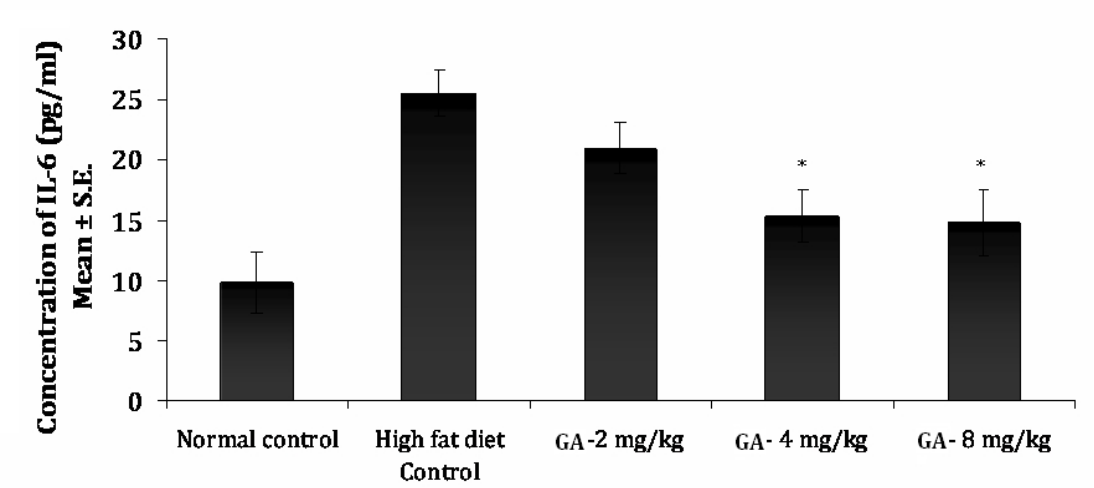

Fig. 6. Expression of IL-6 in serum from high fat diet induced obese C57BL/6 mice treated with different concentration of GA

**= p<0.05; ** = p<0.01; GA: Gallic acid at 2, 4 and 8 mg/kg p.o.; n = 8 per group*

5. DISCUSSION

The present study demonstrates that Gallic acid (GA) isolated from aqueous extract of *Labisia pumila* significantly reduced weight gain in diet induced obese C57BL/6 mice. Before proceeding to the *In vivo* efficacy study on C57Bl/6 mice the in vitro anti-obesity potential of GA was evaluated in mouse 3T3L1 cells. A common assay to measure adipocyte differentiation in cell culture is with the dye Oil Red-O (ORO). During adipocytes differentiation, both LPPM/A003 and GA had inhibitory effect on fat droplet formation and triglyceride accumulation. The concentration at which LPPM/A003 showed 50% inhibition was 94.51µg/ml and that of GA was 19.86µg/ml, however, IC_{50} value was not obtained for PG upto the highest workable concentration (Table 2) (Fig. 2).

GA was then taken up for further investigation for establishing its *In vivo* anti-obesity study. Mice on high fat diet when treated with GA (2, 4 and 8 mg/kg, p. o.) showed a significant decrease in body weight when compared to body weights of mice in high fat diet control group animals (Table 3 and Fig. 3). It was shown that administration of GA at graded doses did not alter food intake in C57BL/6 mice indicating that the prevention of weight gain induced by the test sample is not due to reduction in energy intake (Table 4).

Leptin is a hormone with both central and peripheral effects on metabolism and energy balance. However, in most obese individuals, leptin concentrations are already high because of the increased amount of leptin-secreting adipose tissue [4]. It appears that with increasing leptin concentrations, the hormone induces target cells to become resistant to its actions. Excessive secretion of leptin as in case of obesity or experimental models of induced obesity leads to disrupted functions of hypothalamic centers that an obese subject tends to go on a over feeding mode. Hence, it becomes imperative to bring about effective reduction of the over expressed levels of leptin in obesity and various doses of GA shows promise in this area as indicated in Fig. 4. Significant reduction was observed in serum Triglyceride and VLDL levels at tested dose levels. HDL levels were slightly elevated at 2 and 4mg/kg and significant increase was observed at 8 mg/kg dose and also the elevated levels of LDL was decreased by administration of GA at graded doses (Table 5).

Chronic inflammation appears to be a clinically important change that occurs in adipose tissue when it becomes obese [18] and oral administration of GA at dose levels of 2, 4 and 8mg/kg inhibited the expression of TNF-α and IL-6 in the serum of experimental mice (Figs. 5 and 6). Inflammation is thought to contribute to the development of the sequel of obesity. Adipose tissue is now considered to be an active endocrine organ that secretes various humoral factors (adipokines), and its shift to production of pro-inflammatory cytokines like TNF alpha and IL-6 in obesity likely contributes to the low-level systemic inflammation that is seen in metabolic syndrome-associated chronic pathologies such as obesity. Recent studies have shown that obesity induces chronic local inflammation in adipose tissue, and that cells of the innate immune system, particularly macrophages, are crucially involved in adipose inflammation and systemic metabolic abnormalities. Adipose tissue TNF-α concentration is correlated with obesity [19,20]. TNF-α may increase systemic insulin resistance by promoting the release of fatty acids from adipose tissue into the bloodstream to act on tissues such as muscle and liver [21]. IL-6 expression is also increased in obese adipose tissue; IL-6 expression in adipose tissue from obese individuals is 10-fold that in adipose tissue from lean individuals if normalized for the number of adipocytes present [22]. Plasma concentrations of IL-6 increase with obesity, elevated IL-6 concentration is a predictor for development of type 2 diabetes and for myocardial infarction [23,24]. These data indicate that GA from aqueous extract of *Labisia pumila* appears to be an effective compound capable of modulating weight gain in high fat diet induced obese mice without inducing any adverse effects. These effects appear to be mediated through the reduction of increased leptin levels, raised triglycerides and cholesterol levels and suppression of a low level systemic inflammation.

5. CONCLUSION

The findings demonstrate GA to have a potent anti-obesity activity and this is suggestive of its possible therapeutic usefulness. Its apparent safety over long term administration is encouraging enough to warrant further studies to explore its possible role in modern clinical practice. Optimizing health with the inclusion of nutraceuticals, will allow for more individuals

to be able to control their obesity, and decrease the burden that it may place on them physically, socially, and psychologically.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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