IJPSR (2011), Vol. 2, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 24 July, 2011; received in revised form 14 October, 2011; accepted 25 October, 2011

# AN ETHANOLIC EXTRACT FROM LICORICE (*GLYCYRRHIZA GLABRA*) EXHIBITS ANTI-OBESITY EFFECTS BY DECREASING DIETARY FAT ABSORPTION IN A HIGH FAT DIET-INDUCED OBESITY RAT MODEL

Zafar Ahmad Malik* and Pyare Lal Sharma

Department of Pharmacology, ISF College of Pharmacy, Moga, Gal Kalan- 142001, Panjab, India

**Keywords:**
High Fat Diet,
*Glycyrrhiza Glabra*,
Licorice,
Obesity,
Glucose Tolerance Test,
Lipid Load Test,
Flavonoids

**Correspondence to Author:**

**Zafar Ahmad Malik**

Department of Pharmacology, ISF College of Pharmacy, Moga, Gal Kalan- 142001, Panjab, India

## ABSTRACT

The present study was to investigate the anti-obesity effects of the freeze dried powder of ethanolic extract of Licorice (*Glycyrrhiza Glabra*) (LP) in a rat model of high-fat diet induced obesity and hyperlipidemia. Male wistar rats were fed with a high-fat diet for 8 weeks to induce obesity and hyperlipidemia. High fat diet induced obese rats exhibited increases in body weight gain, adipose tissue mass and levels of serum glucose, triglycerides and total cholesterol compared to rats fed a normal chow diet. However, administration of LP (100-400 mg/kg, o.d., p.o) along with high-fat diet for 8 weeks significantly reduced high fat diet-induced increases in body weight gain and adipose tissue mass in a dose dependant manner. Moreover, LP attenuated high fat diet induced increased levels of serum glucose, triglycerides and total cholesterol. The antiobesity effects of LP were comparable to orlistat, a well reported pancreatic lipase inhibitor. The antiobesity activity of LP appears to be mediated by decreasing dietary fat absorption from the intestine as LP dose dependently attenuated the raising of the serum triglycerides level after oral lipid load test and enhanced excretion of fecal fat content.

**INTRODUCTION:** Obesity is defined medically as a state of increased weight, more specifically adipose tissue, of sufficient magnitude to produce adverse health consequences [1]. It is a serious public health problem throughout the world, affecting both developed societies and developing countries [2]. More than two third of the American population is now considered obese or overweight, and the prevalence of obesity in children is escalating dramatically, signifying even greater medical harm in the decades to come [3, 4].

Patients with obesity have anomalies of blood-glucose homoeostasis, elevated plasma triglycerides (TG) and low levels of high-density lipoprotein (HDL) cholesterol that further contributes to the later appearance of cardiovascular syndromes [5, 6].

Moreover, obese and overweight patients are at higher risk from coronary artery disease, hypertension, hyperlipidemia, diabetes mellitus, cancers, gall bladder disorders, cerebrovascular accidents, osteoarthritis, restrictive pulmonary disease and sleep apnoea [7, 8].

Despite its advance of pandemic proportions, its associated morbidity and mortality, and its financial burden for government and society, obesity remains a major unsolved medical problem [2, 9]. Clinical approaches currently used to control and reduce body weight include lifestyle counseling, pharmacotherapy, and surgery[10]. However, diet and physical exercise are usually not very effective and even after successful weight loss most patients regain weight and bariatric

surgery is reserved for extreme obesity (BMI ≥ 40) because of its cost and complications [11, 12].

Present medications approved for long term obesity treatment are less effective and associated with number of adverse effects some of which are serious in nature [13, 14, 15]. The absence of safe and effective treatments for the obesity has encouraged availability and popularity of natural dietary supplements intended to help with weight loss dramatically in recent years, but therapeutic effectiveness of majority of them remains to be scientifically or medically evaluated [16].

Licorice remains one of the oldest and most commonly prescribed herbs and has been used in the treatment of numerous ailments ranging from tuberculosis to peptic ulcers [17]. Licorice has held claim for therapeutic use for fevers, liver ailments, dyspepsia, gastric ulcers, sore throats, asthma, bronchitis, Addison's disease and rheumatoid arthritis and has been used as a laxative, anti-tussive and expectorant [18, 19]. Recently, interest in licorice has renewed as various reports suggested its beneficial role on body weight in rodents [20, 21, 22].

However, there is paucity in the literature regarding the effectiveness of licorice in obesity and its mechanism of action of anti-obesity activity is poorly understood. Therefore, this study has been designed to investigate the anti-obesity activity of licorice along with its mechanism of action in Wistar rats.

## MATERIALS AND METHODS:

**Plant material and Preparation of Freeze Dried Powder of Licorice:** Roots of licorice purchased from local market were identified and authenticated by the Department of Botany, Punjab University, Chandigarh. The dried plant material was pulverized, coarsely powdered and extracted with 95% alcohol using a Soxhlet extractor.

After exhaustive extraction, the alcoholic extract was filtered, concentrated and completely lyophilized by continuous freeze drying as previously described [23]. The yield was ~7.6 g powder/100g of licorice root powder. The freeze dried powder of licorice (LP) so obtained was stored in airtight container at low temperature and suspended in 0.5% carboxymethylcellulose (CMC) before oral administration.

**Phytochemical Screening and Standardization:** Phytochemical screening of the MC was carried out employing standard procedures and tests [24]. One gram of LP was weighed accurately and suspended in 100 ml of water. The mixture was vortexed (3 min) followed by ultrasonication (30 min) and then centrifuged at 1500×g (10 min) to get supernatant sample solution for total phenolic content, total flavonoid. For total phenolic content, the sample solution (200 mL) was added to 800mL of 7.5% sodium carbonate solution, 1mL of Folin-Ciocalteu reagent was added, and the mixture was left to stand for 30 min. The absorbance was measured at 765nm using UV 1700, Shimadzu spectrophotometer.

The total phenol content was expressed as micromoles of gallic acid equivalent in 1 g of LP [25]. For total flavonoid content 0.5 ml of sample solution was mixed with 2ml of distilled water and subsequently with 0.15 ml of $NaNO_2$ (5%) solution. After 6 min of incubation, 0.15 ml of $AlCl_3$ (10%) solution was added and then allowed to stand for 6 min, followed by adding 2 ml of sodium hydroxide (4%) solution to the mixture. Water was added immediately to the sample to bring the final volume to 5 ml, the mixture was thoroughly mixed, allowed to stand for another 15 min and measured spectrophotometrically at 510 nm. The total flavonoid content was estimated using a standard curve with quercetin and expressed as g of quercetin equivalents in 1 g of LP [26].

**Acute Toxicity Study:** For acute toxicity study 18 h fasted animals were divided into several groups of 10 rats each. The LP in the dose range of 100–4000 mg/kg was administered orally to different groups. The animals were examined continuously for 3 h and then occasionally for further 4 h; finally, overnight mortality if any was recorded [27].

**Preparation of High fat diet:** Obesity was induced by high-fad diet, prepared by mixing 33% normal chow (Ashirwad Diets, Punjab, India), 33% Nestle milk powder, 7% sucrose and 27% tap water by weight [28]. This diet provides 68% energy as carbohydrate, 20% as protein and 12% as fat and is reported to produce reliable weight gain (obesity) in Wistar rats while as

normal chow (NC) provides 65% of energy as carbohydrate, 20% as protein and 4% as fat [29].

**Animal Treatments:** Male Wistar rats of 7-8 weeks of age were procured from the animal facility of the Institute. The animals were housed in standard polypropylene cages (two rats/cage) and maintained under controlled room temperature (25±2$^{o}$C) with 12:12 h light and dark cycle. The guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Govt. of India were followed and prior permission was sought from the institutional animal ethics committee for conducting the study.

Animals were randomized on the basis of their body weight and divided into various groups (n=10). Normal control group was fed NC and high fat control group was fed high-fad diet for 8 weeks. The low LP, medium LP and high LP groups were administered with LP at a dose of 100, 200 and 400mg/kg, respectively, for 8 weeks. The orlistat group was administered with orlistat at a dose of 30mg/kg. LP and orlistat were orally administered daily to the rats by gavage while as control groups received vehicle every day by same route.

Except normal control, all the groups were continually fed the high-fat diet during the experiment. All the animals had free access to water and the animals were inspected daily. Food intake and body weight were measured twice weekly. Feces of rats were collected on three consecutive days at the beginning of 6th week for the determination of total fat content. One week before the sacrifice, animals were subjected to oral glucose tolerance test (OGTT). At the end of the stipulated period, blood for various biochemical parameters was obtained retrorbitally under light ether anesthesia and the animals were sacrificed by cervical dislocation.

The blood was collected into tubes, serum separated and analyzed on the same day. The gastrocnemius muscle, epididymal, mesenteric and retroperitoneal white adipose tissue (WAT) were dissected, cleaned of, weighed and stored in 10% buffered formalin solution. Lee index [30] i.e., (Body Wt in gms)$^{1/3}$ / (ano-nasal length in cm), an index of obesity, was calculated at the end of the experiment.

**Histological Analysis and Morphometry**: Epididymal adipose tissue was fixed in 10% formalin and then embedded with paraffin. Tissue sections (10 µm) were cut and mounted on microscope slides. After being air-dried, they were stained with hematoxylin and eosin and photographed at 100X magnification. At least two fields per slice and six slices per fat mass were analyzed for the purpose of quantifying adipocyte size.

**Oral Lipid Load Test**: Normal Wistar rats were randomly divided into five groups (n=6). A lipid emulsion was prepared with 7ml of olive oil; 93 mg of cholic acid and 7ml of distill water[31]. After fasting for 24 h, male Wistar rats were orally administrated by the lipid emulsion (final concentration 10 ml/kg body weight) and different doses of LP, orlistat (30mg/kg) or vehicle. Blood samples were taken from retro-orbital plexuses at 0, 1, 2 and 3 h after administration of emulsion using a capillary tube, and centrifuged at 8000 rpm for 10 min. to obtain the serum. Control rats received 0.5% CMC along with lipid emulsion. Serum triglyceride level was measured using a commercially available kit (Tulip Diagnostic (P) Ltd, Goa, India).

**Measurements:** Serum glucose, triglyceride, total cholesterol and HDL cholesterol, SGOT and SGPT concentrations were measured by using commercially available kits (Tulip Diagnostic (P) Ltd, Goa, India). During OGTT glucose levels were quantified at the start (t = 0), 30, 60, 90 and 120 min after the administration of the glucose load (2g/kg). The total fat content in feces was determined gravimetrically. The samples were dried (105°C for 12 h) and then extracted with petroleum ether under reflux [32].

**Statistics:** All values are expressed as mean ± standard deviation (S.D). Glucose responses during the glucose tolerance test were evaluated by estimation of the total area under the curve ($AUC_{0\text{-}2hr}$), using the trapezoidal method. The significance of the differences between the means of various groups was established by one way ANOVA with a Tukey's post hoc test using the Graphpad Prism 4 software. The p value < 0.05 was considered to be statistically significant.

**RESULTS:**

**Phytochemical screening and determination of Polyphenols and Flavonoids:** Qualitative phytochemical screening of the LGP showed the

presence of bioactive principles such as phenolic compounds, alkaloids, tannins, glycosides, saponins, steroids, flavonoids, carotenoids, among others. The total phenolic and flavonoids were estimated to be 145.7 ± 12.1 mg gallic acid equivalents/g of the LP and 89.6 ± 9.1 mg of quercetin equivalents/g of LP, respectively.

**Acute Toxicity Study:** No treatment- related signs of toxicity or mortality in any of the animals were observed. The three doses 100, 200 and 400 mg/kg used in the present study correspond to 2.5, 5 and 10 % of no-observed-adverse-effect level (NOAEL) of the LP (4000 mg/kg), respectively.

**Effect of LP on Body Weight Adipose Tissue Weight and Lee's Index:** Obesity was induced in normal rats by feeding a high-fat diet for 8 weeks. The mean body weights of the six experimental groups were similar at the start of the experiment. After 8 weeks, rats fed a high-fat diet had 21.5% higher body weights compared to NC diet-fed control group ($p<0.05$). Adipose tissue mass was also significantly increased by 142% in high-fat diet-fed rats compared to normal chow-fed animals ($p<0.05$). In contrast, LP-treated groups continued to gain weight at a slower rate than the high fat control group. After 8 weeks of the treatment, the rats administered low dose LP (LP-L), medium dose LP (LP-M) and high dose LP (LP-H) had gained 1.7% ($p>0.5$), 8.2% ($p>0.5$) and 13.6% ($p< 0.5$) less body weight than the high fat fed-control group respectively (Table 1).

Similarly, high fat diet induced increase in adipose tissue weights were 12.5% ($p>0.5$), 22.6% ($p< 0.5$) and 39.3% ($p< 0.5$) reduced compared to the high fat control rats by LP-L LP- M and LP-H treatment, respectively. On the other hand, the body weight and adipose tissue weight in orlistat-treated group were 14.9% ($p<0.5$) and 30.5% ($p<0.5$) respectively decreased compared to high fat control animals. Compared with the normal control group Lee's index was significantly increased in high fat fed animals and was significantly reduced in rats fed a high-fat diet plus LP or orlistat groups compared to the high fat diet animals (**Table 1**).

**TABLE 1: EFFECT OF LP ON BODY WEIGHT, ADIPOSE TISSUES WEIGHT AND VARIOUS BIOCHEMICAL PARAMETERS IN RATS FED A HIGH-FAT DIET FOR 8 WEEKS**

| Parameters | NC | HFD-C | Licorice (mg/kg) | | | Orlistat (30mg/kg) |
|---|---|---|---|---|---|---|
| | | | 100 | 200 | 400 | |
| **Initial Body Wt (g)** | 159 ± 13.8 | 157.4 ± 18.3 | 153.4±13.53 | 156.8±14.1 | 157.8±13.8 | 155.6±12.8 |
| **Final Body Wt (g)** | 265 ± 8.2 | 322.5 ±36[a] | 316.8±38.5 | 296±28.3 | 278.6±24.3[b] | 274.4±22.6[b] |
| **Lee index** | 0.27± .01 | 0.28± 0.01[a] | 0.28±0.01 | 0.27±0.01[b] | 0.26±0.01[b] | 0.26±0.01[b] |
| **Energy Intake (kcal/day)** | 64± 4.8 | 79 ± 8.1[a] | 80.2±9.4 | 79.4±9.8 | 81.6±11.8 | 83.7±12.7 |
| **Fecal fat (g/day)** | 0.06 ±0.02 | 0.31± 0.03[a] | 0.48±0.03[b] | 0.66±0.05[b] | 0.72±0.05[b] | 0.55±0.06[b] |
| **WAT (g)** | 8.9±1.3 | 21.6±3.6[a] | 18.9±3.9 | 16.7±3.9[b] | 13.1±2.03[b] | 15±1.9[b] |
| **Epididymal** | 2.6± 1.3 | 6.6± 1.7 | 6.9±3 | 6±2.5 | 4.5±1.4 | 4.8±1.8 |
| **Retroperitoneal** | 3.3±0.8 | 8.9 ± 1.7 | 6.5±1.9 | 6.2±3.5 | 4.5±1.2 | 6.4±2.8 |
| **Mesenteric** | 2.9±0.9 | 6± 0.8 | 5.5±1.8 | 4.6±1.7 | 4.1±1.7 | 3.8±1.3 |
| **Gastrocnemius mass (g)** | 1.9±0.3 | 2.1± 0.2 | 2.1±0.1 | 2.1±0.3 | 2.2±0.2 | 2.1±0.2 |
| **Glucose (mg/dl)** | 93.8± 20.1 | 131.6±20.1[a] | 103.7±19.1[b] | 100.2±18.6[b] | 95.1±10.8[b] | 105.8±17.3[b] |
| **OGTT ($AUC_{0-2hr}$)** | 14558±1400 | 18219±2055[a] | 16935±1400 | 16228±1600 | 15755±1720[b] | 16005±1175 |
| **TG (mg/dl)** | 49.9 ±13.4 | 119.8±24.9[a] | 94.8±18[b] | 86.6±13[b] | 81.4±9.9[b] | 90.4±11.3[b] |
| **TC (mg/dl)** | 54.3 ±11.7 | 81.6± 13.9[a] | 79.1±13.8 | 70.1±12.5 | 61.0±6.8[b] | 62.8±12.2[b] |
| **HDL (mg/dl)** | 23.46±5.1 | 29.14 ± 4.9[a] | 29±6.8 | 28.7±6.5 | 31.2±5.9 | 30.7±5.4 |
| **AST (IU/L)** | 62.7±20.5 | 83.4± 13.4 | 74.1±16.4 | 64.8±11.1[b] | 60.9±8.8[b] | 79.1±9.8 |
| **ALT (IU/L)** | 32.5±4.5 | 35.2± 9.3 | 33.8±8.5 | 30.6±8.7 | 30.3±8.6 | 36.2±7.2 |

Results: mean ± SD. a= $p<0.05$ vs NC (Normal Control); b= $p<0.05$ vs HFD-C (High Fat Diet Control)

**Effect of LP on average daily energy intake and fecal fat content:** The food intake per day per rat was weighed in all groups for calculation of energy intake. The daily energy intake was analyzed by average for 8 weeks. The average daily energy intake of each animal over the experimental period is illustrated in Table 1. The rats in the high fat control group consumed significantly more energy than the normal chow fed rats and subsequently excreted higher fecal fat content. The daily energy intakes in LP or orlistat-treated groups were not significantly different compared to high fat fed control in all study period. However average daily fecal fat content of rats on LP groups were significantly increased compared to high fat fed control in a dose dependant manner indicating reduced apparent fat absorption. The fecal fat content of orlistat group was also significantly increased compared to high fat diet control group.

**Effect of LP on Serum Biochemical Parameters:** Compared to a normal chow diet, high-fat diet produced significant hyperglycemia (40%), hypertriglyceridemia (140%) and hypercholesterolemia (50%). However, administration of LP for 8 weeks caused significant reductions in serum glucose, triglyceride and total cholesterol levels in a dose dependant manner, compared to high-fat diet control (Table 1). This suggests that LP treatment suppresses biochemical disorders found in high-fat diet-fed obese rats, thereby preventing their hyperglycemia, hypertriglyceridemia and hypercholesterolemia. Orlistat also significantly decreased high fat diet induced increase in serum glucose, triglyceride and total cholesterol.

**Effect of LP on Oral Glucose Tolerance Test (OGTT):** There were no significant changes in the fasting glucose concentrations in the normal and high-fad diet control indicating high-fad diet did not produce frank diabetes (data not shown). However, the glucose tolerance ability of the high-fad diet control group was significantly lower than that of the NC group. In contrast, LP significantly prevented high-fat diet induced glucose intolerance in a dose dependent manner but the results were statistically significant only in case of high dose of LP. Orlistat also attenuated high fat diet induced glucose tolerance but did not reach statistically significant level (**Fig. 1**).

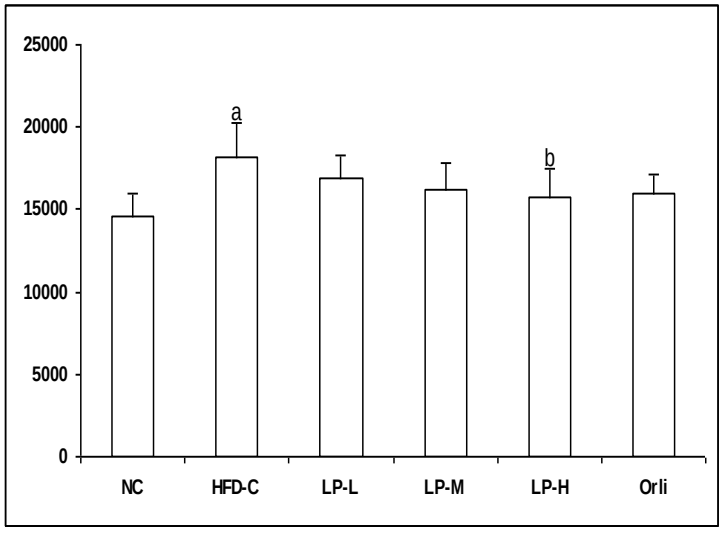


**FIG. 1: EFFECT OF LP ON OGTT IN RATS FED A HIGH-FAT DIET FOR 8 WEEKS**

Values are expressed as total AUC ± S.D of OGTT. a=p< 0.05 Vs NC; b=p< 0.05 Vs HFD-C. NC: Normal Control; HFD-C: High Fat Diet Control; LP-L: Licorice 100mg/kg treated animals; LP-M: Licorice 200mg/kg treated animals; LP-H: Licorice 400mg/kg treated animals; Orli: Orlistat 30 mg/kg treated animals.

**Effect of LP on Adipocyte size:** Histological examination of epididymal WAT revealed that high-fat diet fed rats had markedly increased adipocyte size (**Fig. 2**) than did normal chow-fed rats. LP or orlistat markedly suppressed epididymal adipocyte size compared to high-fat diet fed rats (**Fig. 3**).

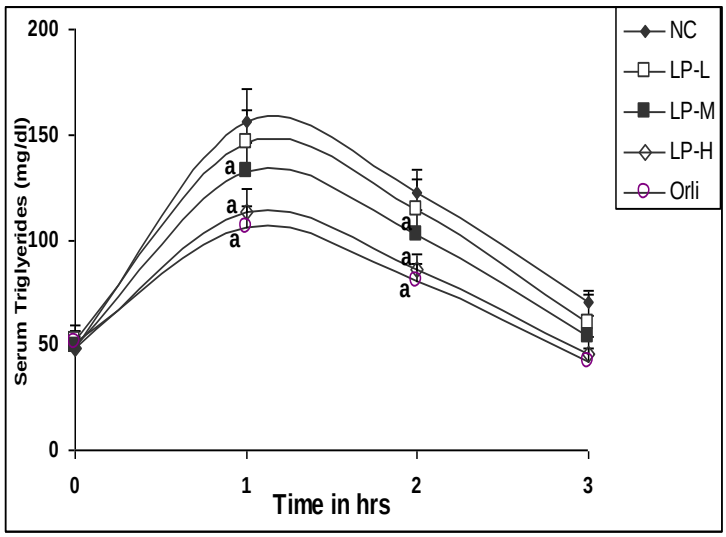


**FIG. 2: EFFECT OF LP ON SERUM TRIGLYCERIDE LEVELS AFTER ORAL LIPID LOAD TEST IN NORMAL RATS**

Values are mean ± S.D.a=p<0.05 Vs NC; NC: Normal Control;LP-L: Licorice 100 mg/kg treated animals; LP-M: Licorice 200mg/kg treated animals; LP-H: Licorice 400mg/kg treated animals; Orli: Orlistat 30 mg/kg treated animals

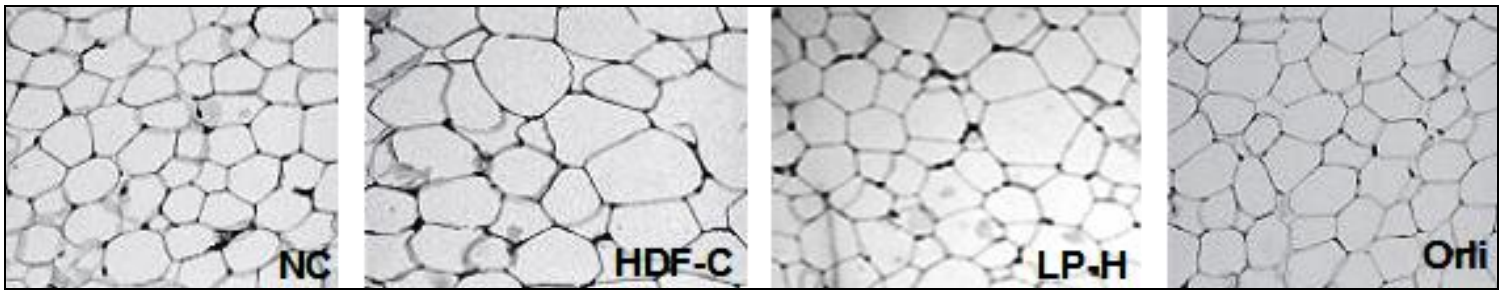


**FIG 3: HISTOLOGICAL CHANGES IN EPIDIDYMAL ADIPOSE TISSUE.** Representative sections of Hematoxylin-Eosin stained epididymal WAT from Normal control (NC), High Fat Diet control (HFD-C), Licorice 400 mg/kg (LP-H) and Orlistat 30 mg/kg (Orli) treated animals (open magnification, X100)

**Effect of LP on Fat Absorption in Normal Rats after a Single Oral Administration of Lipid Emulsion:** For the purpose of clarifying the mechanism of body weight reduction the effect of LP on absorption of triglycerides in the intestine was investigated by a lipid loading test in normal rats. The serum triglyceride level at 1 h after oral administration of a lipid emulsion to normal rats was elevated considerably and was significantly ($p < 0.05$) decreased in the groups treated with medium and high doses of LP and orlistat (Fig. 2). The results suggest that LP has pancreatic lipase inhibitory activity akin to orlistat.

**Effect of LP on Liver Function and Lean tissue mass:** The effect of high fat diet and different treatments on liver function is shown in Table 1. High fat diet produced slightly modifications of the AST levels indicating slight damage to the liver but did not reach statistically significant level. However, LP treatment produced significant decrease in AST levels compared to high fat fed animals indicating protective effect of LP against high fat diet induced liver damage. On the other hand orlistat did not exhibit any significant change in liver function. Furthermore LP or orlistat did not produce any change in gastrocnemius muscle weight, an index of lean tissue mass, indicating LP or orlistat promotes loss of body fat rather than lean tissue mass (Table 1).

**DISCUSSION:** We examined the anti-obesity effect of LP in high-fat diet-fed rats, since this metabolic model of obesity reproduces human obesity better than the genetic obese models. In the present study Wistar rats fed a high-fat diet had significant increased body weight, adipose tissue weight and levels of serum glucose, triglycerides and total cholesterol compared to rats fed a normal chow diet.

Treatment with LP not only reduced the body weight, various adipose pad weights and Lee's index, but also attenuated high-fad diet induced increase in serum TC, TG and glucose levels. The anti-obesity activity of LP appears partly to be mediated by decreasing dietary fat absorption from the intestine via inhibition of pancreatic lipase activity.

Obesity is a multifactorial disease that develops from the interaction between genotype and environment [33]. Although genetic factors can contribute to the development of obesity, the rapid surge in the global prevalence of obesity suggest that common environmental and lifestyle factors may be promoting and exacerbating the problem [33]. The consumption of high fat diet is thought to be one of the main factors [34]. Dietary fat is calorically dense and extremely palatable. It is easily over consumed because it can cause less satiety than carbohydrate and protein [35]. Moreover, ingestion of fat does not acutely stimulate fat oxidation or energy expenditure, but rather, it promotes fat storage [36].

Therefore, a high fat diet can lead to hyperphagia, weight gain and increased adiposity. Moreover, high fat dietary obesity is the simplest obesity induction model and possibly the one that closely resembles the reality of obesity in humans [37]. Based on this model, the anti-obesity effect of LP in rats fed a high-fat diet was investigated by analyzing the changes in body weight, adipose tissue weight and blood biochemicals.

A high fat diet not only lowers glucose uptake but also inadequately suppresses hepatic glucose production stimulated by insulin leading to insulin resistance as well as hyperglycaemia [38]. High fat diet also alters both basal and stress induced hypothalamic pituitary adrenal activity to increase adrenal glucocorticoid

production in rats [39]. Elevated glucocorticoids can subsequently lead to hypertriglyceridemia by decreasing the level of lipoprotein lipase [40]. Thus, the observed high fat diet induced increased levels of serum glucose, triglycerides and total cholesterol may be due to insulin resistance and combination of above effects. Licorice or its active constituent, glycyrrhitinic acid, (GA) has been reported to reduce body fat mass, however, prolonged administration of licorice or GA can produce hypokalemic hypertension [41, 42].

The non-aqueous fraction of licorice which mainly contains flavonoids in high proportion but GA in trace amount improved blood glucose level, reduced accumulated abdominal fat, decreased adipocyte size, improved fatty liver condition and upregulated genes for beta oxidation and down regulated those for fatty acid synthesis in high fat induced obese mice [20, 21, 22]. The non-aqueous fraction of licorice appears to have more appealing anti-obesity potential; therefore, the ethanolic extract of licorice was employed in the present study in Wistar rats.

The anti-obesity effect of LP on high-fat diet was thoroughly studied. It was observed that the fecal fat content of the LP treated animals increased in a dose dependant manner suggesting reduced apparent fat absorption. To confirm the hypothesis that prevention of obesity by LP is caused by reduced lipid absorption, the effect of LP on absorption of triglycerides in the intestines was investigated by lipid load test. LP decreased the serum triglyceride gain caused by lipid load suggesting inhibition of lipid from intestines.

It is well reported that dietary fat is absorbed by the intestine only after the fat has been subjected to the action of pancreatic lipase and converted to chylomicrons composed mainly of triglycerides and cholesterol [43]. It is also reported that lipids stored in adipose tissue are largely derived from circulating triglycerides derived from these chylomicrons and the remnants of the chylomicrons ultimately circulate in the blood stream in the form of cholesterol [44, 45].

Thus, it may be suggested that decreased dietary fat absorption from the intestine via inhibition of pancreatic lipase activity by LP may be responsible for attenuation of high fat diet induced body weight gain, adipose tissue weight and hyperlipidemia.

This contention is supported by the observation that LP treatment significantly increased fecal fat content in obese rats in a dose dependant manner. A decrease in the size of the adipocyte further support the argument that LP suppresses high fat diet induced fat accumulation. Moreover, the changes in body weight suggest that decreases in body weight may be attributed to decreases in body fat rather than lean tissue. Both the changes in the weight of the visceral fat pads and no significant change in gastrocnemius muscle weight are supportive of this interpretation.

The beneficial effect of high dose of LP in preventing the high fat diet induced body weight gain has been observed to be almost similar to the effect produced by orlistat, well reported pancreatic lipase inhibitor. But, the LP was noted to be slightly superior in attenuating the high fat diet induced increase in hyperglycemia. LP has been reported to have beneficial effect on insulin resistance by decreasing insulin level even in case of feeding high fat diet [46].

Thus, the better attenuation of high fat diet induced hyperglycemia by LP may be due to reduction of insulin resistance and subsequent decreased insulin level. This contention is supported by the fact that the high fat diet induced glucose intolerance was noted to be significantly reduced by treatment with high dose of LP. On the other hand, the results demonstrated that there were no general toxicities associated with LP treatment. In fact, licorice was found to be hepatoprotective against high fat diet induced liver damage. This observation is in keeping with the hepatoprotective effect of licorice and its constituents [47].

**CONCLUSION:** On the basis of above discussion, it may be concluded that LP attenuated high fat diet induced increase in the body weight, visceral adipose pad weights and Lee's index, serum TC, TG and glucose levels. The anti-obesity activity of LP appears partly to be mediated by decreasing dietary fat absorption from the intestine.

**REFERENCES:**

1. Spiegelman BM and Flier JS: Obesity and the Regulation of Energy Balance. Cell 2001; 104:531–543.
2. Arora S and Anubhuti: Role of neuropeptides in appetite regulation and obesity–a review. Neuropeptides 2006; 40:375–401.

3. Troiano RP and Flegal KM: Overweight prevalence among youth in the United States: why so many different numbers? Int. J. Obes. Relat. Metab. Disord. 1999; 2: S22–S27.
4. Flier JS: Obesity wars: molecular progress confronts an expanding epidemic. Cell 2004; 116: 337–350.
5. Despres JP, Lemieux I and Almeras N: Contribution of CB1 blockade to the management of high-risk abdominal obesity. International Journal of Obesity 2006; 30: S44–S52.
6. St-Pierre J, Lemieux I, Perron P, Brisson D, Santure M, Vohl MC, Despres JP and Gaudet D: Relation of the "hypertriglyceridemic waist" phenotype to earlier manifestations of coronary artery disease in patients with glucose intolerance and type 2 diabetes mellitus. American Journal of Cardiology 2007; 99: 369–373.
7. Flegal KM, Carroll M., Ogden CL and Johnson CL: Prevalence and trends in obesity among US adults, 1999-2000. *JAMA* 2002; 288: 1723-1727.
8. Eckel RH, York DA, Rossner S, Hubbard V, Caterson I, Jeor ST, Hayman LL, Mullis RM and Blair SN: American Heart Association. Prevention Conference VII: obesity, a worldwide epidemic related to heart disease and stroke: executive summary. Circulation 2004; 110: 2968–2975.
9. Kopelman PG: Clinical treatment of obesity: are drugs and surgery the answer? Proc. Nutr. Soc. 2005; 64:65–71.
10. Fernstrom JD and Choi S: The development of tolerance to drugs that suppress food intake. Pharmacology & Therapeutics 2008; 117:105–122.
11. Glazer G. Long-term pharmacotherapy of obesity 2000. A review of efficacy and safety. Arch Intern Med 2001; 161:1814 – 1824.
12. Blackburn GL: Solutions in weight control: lessons from gastric surgery. Am. J. Clin. Nutr. 2005; 82(Suppl. 1):248S-252S.
13. Bray GA: Drug treatment of obesity. Don't throw out the body with bath water. Am J Clin Nutr. 1998; 67: 1-2.
14. Li Z, Maglione M, Tu W, Mojica W, Arterburn D, Shugarman LR, Hilton L, Suttorp M, Solomon V, Shekelle PG and Morton SC: Meta-analysis: pharmacologic treatment of obesity. Ann. Intern. Med. 2005; 142:532–546.
15. Bray GA and Tartaglia. LA: Medicinal strategies in the treatment of obesity. Nature 2000; 404:672-677.
16. Han LK, Xu BJ, Kimura Y, Zheng Y and Okuda H. Platycodi radix affects lipid metabolism in mice with high fat diet-induced obesity. J Nutr 2000; 130:2760-2764.
17. Huang KC: The Pharmacology of Chinese Herbs. CRC Press, Inc, Boca Raton, FL. 1993; 275–278.
18. Anon: Glycyrrhiza glabra. Alternative Medicine Review 2005;10: 230–237.
19. Wang ZY, Athar M and Bickers DR:. Licorice in foods and herbal drugs: Chemistry, pharmacology, toxicology and uses. In: Mazza, G., Oomah, B.D. (Eds.), Herbs, Botanicals & Teas. Technomic Publishing Co. Inc, Lancaster, PA, 2000; 321–353.
20. Mae T, Kishida H, Nishiyama T, Tsukagawa M, Konishi E, Kuroda M, Mimaki Y, Sashida Y, Takahashi K, Kawada T, Nakagawa K and Kitahara M:. A licorice ethanolic extract with peroxisome proliferator activated receptor γ ligand binding activity affects diabetes in KK-Ay mice, abdominal obesity in diet induced obese C57BL mice and hypertension in spontaneously hypertensive rats. *J Nutr*, 2003; **133**:3369-3377.
21. Nakagawa K, Kishida H, Arai N, Nishiyama T and Mae T: Licorice flavonoids suppress abdominal fat accumulation and increase in blood glucose level in obese diabetic KK-Ay mice. Biol Pharm Bull, 2004; **27**: 1775-1778.
22. Aoki F, Honda S, Kishida H, Kitano M, Arai N, Tanaka H, Nakagawa K, Asakura T, Nakai Y and Mae T: Suppression by licorice flavonoids of abdominal fat accumulation and body weight gain in high fat diet induced obese C57BL/6J mice. Biosci Biotechnol Biochem. 2007; **71**:206-214.
23. Malik ZA, Singh M and Sharma PL: Neuroprotective effect of Momordica charantia in global cerebral ischemia and reperfusion induced neuronal damage in diabetic mice. J Ethnopharmacol 2011; 133:729-734.
24. Trease GE and Evans WC: 1989. Pharmacognosy, 13th ed. Bailliere Tindall, London.
25. Singleton VL and Rossi JAJ: Colorimetric of total phenolics with phosphomolybdic–phosphotungstic acid reagents. American Journal of Enology and Viticulture 1965; 16: 144–158.
26. Zou YP, Lu YH and Wei DZ: Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum* L. in vitro. Journal of Agricultural and Food Chemistry 2004; 52:5032–5039.
27. Litchfield JT and Wilcoxon F: A simplified method of evaluating dose–effect experiments. Journal of Pharmacology and Experimental Therapeutics 1949; 96: 99–113.
28. Wilding JP, Gilbey SG, Mannan M, Aslam N, Ghatei MA and Bloom SR: Increased Neuropeptide Y Content In Individual Hypothalamic Nuclei, But Not Neuropeptide Y Mrna, In Diet-Induced Obesity In Rats. Journal of Endocrinology 1992; 132: 299-304.
29. Brown M, Bing C, King P, Pickavance L, Heal D and Wilding J: Sibutramine reduces feeding, body fat and improves insulin resistance in dietary-obese male Wistar rats independently of hypothalamic neuropeptide Y. British Journal of Pharmacology 2001;132: 1898 – 1904.
30. Bernardis LL and Patterson BD: (1968). Correlation between `Lee index' and carcass fat content in weanling and adult female rats with hypothalamic lesions. J Endocrino.1968; 40:527-528.
31. Ohkoshi E, Miyazaki H, Shindo K, Watanabe H, Yoshida A and Yajima H: Constituents from the leaves of *Nelumbo nucifera* stimulate lipolysis in the white adipose tissue of mice. Planta Med. 2007; **73**:1255-1259.
32. Chen Q, Chan LL and Li ET: Bitter melon (*Momordica charantia*) reduces adiposity, lowers serum insulin and normalizes glucose tolerance in rats fed a high fat diet. J Nutr 2003; 133:1088–1093.
33. Goran MI and Treuth MS: Energy expenditure, physical activity, and obesity in children. Pediatr Clin North Am. 2001; 48: 931–953.
34. Woods SC and Seeley RJ: Understanding the physiology of obesity: review of recent developments in obesity research. International Journal of Obesity 2002; 26: Suppl 4:S8-S10.
35. Rolls BJ and HammerVA: Fat, carbohydrate, and the regulation of energy intake. American Journal of Clinical Nutrition 1995; 62:1086S–1095S.
36. Flatt JP, Ravussin E, Acheson KJ and Jequier E: Effects of dietary fat on postprandial substrate oxidation and on carbohydrate and fat balances. J Clin Invest. 1985; 76(3):1019–1024.
37. Buettner R, Scholmerich J, Bollheimer LC: High-fat Diets: Modeling the Metabolic Disorders of Human Obesity in Rodents. Obesity 2007; 15: 798-808.
38. Oakes ND, Cooney GJ, Camilleri S, Chisholm DJ and Kraegen EW: Mechanisms of liver and muscle insulin resistance induced by chronic high-fat feeding. Diabetes. 1997; 46:1768–1774.
39. Tannenbaum BM, Brindley DN, Tannenbaum GS, Dallman MF, McArthur MD and Meaney MJ: High-fat feeding alters both basal and stress-induced hypothalamic-pituitary adrenal activity in the rat. Am J Physiol. 1997; 273(6 Pt 1): E1168–E1177.
40. Mantha L, Palacios E and Deshaies Y: Modulation of triglyceride metabolism by glucocorticoids in diet-induced obesity. Am J Physiol. 1999; 277(2 Pt 2):R455–R464.

41. Armanini, D., Lewicka, S., Pratesi, C., Scali, M., Zennaro, M.C., Zovato, S., Gottardo C, Simoncini M, Spigariol A, Zampollo V: Further studies on the mechanism of the mineralocorticoid action of licorice in humans. J Endocrinol Invest.1996; 19: 624-629.
42. Armanini D, De Palo CB, Mattarello MJ, Spinella P, Zacearia M, Ernaolao A., Palermo M, Fiore C, Sartorato P, Francini-Pesenti F, Karbowiak I: Effect of liqorice on the reduction of body fat mass inn healthy subjects. J Endocrnol Invest. 2003; 26**:**646-650.
43. Lowe ME: Pancreatic triglyceride lipase and colipase: insights into dietary fat digestion. Gastroenterology 1994; 107: 1524–1536.
44. Lupien P, Brun D, Gagne C, Moorjani S, Bielman P and Julien P:. Gemfibrozil therapy in primary type II hyperlipoproteinemia: effects on lipids, lipoproteins and apolipoproteins. The Canadian Journal of Cardiology 1991; 7: 27–33.
45. Fruchart JC, Brewer Jr HB and Leitersdorf E, Consensus for the use of fibrates in the treatment of dyslipoproteinemia and coronary heart disease. Fibrate Consensus Group. The American Journal of Cardiology 1998; 81: 912–917.
46. Takki H, Kometani T, Nishimura T, Nakae T, Okada S and Fushiki T: Antidiabetic effect of glycyrrhizin in genetically diabetic KK-Ay mice. Biological & Pharmaceutical Bulletin 2001; 24: 484–487.
47. Nagai T, Egashira T, Yamanaka Y and Kohno M: The protective effect of glycyrrhizin against injury of the liver caused by ischemia-reperfusion. Arch Environ Contam Toxicol 1991; 20: 432–436.

**********