Chirag A. Patel*, Jalaram H. Thakkar, Priyanka Patel, Devdas Santani

1Department of Pharmacology, SSR College of Pharmacy, Silvassa, Dadra and Nagar Haveli, India-396230.
2Department of Pharmacology, ROFEL, Shri. G. M. Bilakhia College of Pharmacy, Vapi, Gujarat, India-396191.
3Department of Pharmaceutics, SSR College of Pharmacy, Silvassa, Dadra and Nagar Haveli, India-396230.

ABSTRACT
The present study examined the anti-obesity effects of *Pinus sylvestris* bark (PS). Anti-obesity activity of PS (286 and 667 mg/kg/day for 56 days) evaluated using High fat diet induced obesity (58.23 % of energy as Fat) in C57BL/6J female mice. We also investigated the effect of PS extract on adipocyte differentiation of 3T3-L1 preadipocytes and in-vitro pancreatic lipase inhibitory activity. Oral treatment with PS ethanol extracts for a period of 56 days, resulted in significant precluded the increase in body weight, adipose tissue weight and serum triglycerides, total cholesterol, leptin and insulin levels in mice with obesity induced by a high-fat diet. PS extract showed the highest and significant inhibitory effect (28.00 ± 0.86 %) at concentration of 250 μg/ml on pancreatic lipase activity. The results of in vitro cytotoxicity assay also showed that PS efficiently inhibited the proliferation in 3T3-L1 preadipocytes. In conclusion, *Pinus sylvestris* bark can be used for an obesity preventing or therapeutic agent because the extract can suppress the adipocyte differentiation, decrease body weight and body fat in an obese animal and inhibition of intestinal lipid absorption thereby suppressing fat accumulation.

KEYWORDS: Pinus Sylvestris, Obesity, Leptin, Pancreatic lipase.
INTRODUCTION
Obesity in general is defined as the presence of excess adipose tissue in the body to such a degree that it may lead to health hazards.[1][2] In developed and developing countries, overweight and obesity are most prevalent nutritional problems. The world health organization has described obesity as one of today's most neglected public health problems, affecting very region of the globe.[3] Overweight and obesity are not only associated with an increased burden of diabetes, hypertension, but also some type of cardiovascular diseases, cancers and premature, social and psychological effects of excess weight.[4] Owing to the adverse side effects associated with many anti-obesity drugs, more recent drug trials have focused on screening for natural sources that have been reported to reduce body weight and that generally have minimal side effects.[5] Although the public enthusiasm for herbal remedies is growing around the world as well as in the Far East, the lack of scientific analysis published on the herbs makes it difficult to determine both the validity of their effects and their mechanisms of action. Due to the limited data concerning the efficacy of herbs and mechanistic explanation of their effects, more detail herbal studies are demanding.

*Pinus sylvestris* is an evergreen coniferous tree growing up to 25 m in height and 1 m trunk diameter.[6] Phytochemical information regarding Pine exposed of following constituents: Flavonoid[7], Essential oil[8] while the Merck Index (1968) lists dipentene, pinene, sylvestrene, cadinene and 3-35% bomyl acetate.[9] Phenolic acid (dihydroxybenzoic acid), unsaturated fatty acids, saturated fatty acids, resin acids (pimaric, dehydroabietic acid and abietic acids), and sterol (sitosterol).[10] According to commercial sources who market weight loss products, the pinolenic acid contained in pine nut oil can help limit appetite.[11] However, it has not yet been reported whether *Pinus sylvestris* affects body weight gain in obesity models. The present investigation designs to determine effect of *Pinus sylvestris* bark on body weight in an obese animal model.

MATERIALS AND METHODS
Collection and Extraction of plant material
The bark of *Pinus sylvestris* was collected from Hareshwar Mill, India and deposited in our laboratory. The bark of *Pinus sylvestris* was shade dried removal of any visible wood material and reduced to coarse powder using a knife mill. The powdered material obtained was then subjected to extraction using 80% v/v ethanol as a solvent in a Soxhlet extractor.
The extract obtained was evaporated at 100 °C to get a semisolid mass. The extracts thus obtained were subjected to phytochemical analysis.[12]

**Phytochemical estimation**

The extract of bark of *Pinus sylvestris* were subjected to analysis for the various phytoconstituents.[13] Total soluble phenolics in the extracts were determined with Folin-Ciocalteu reagent according to the method of Singleton[15] (1977) using Gallic acid as a standard phenolic compound. Total soluble Flavonoid Content in the extracts was determined according to colorimetric aluminum chloride method.[16]

**Acute toxicity studies**[17]

The acute oral toxicity study of PS was performed according to the OECD (organization for economic co-operation and development) guideline 420.

**Cell lines and culture**

3T3-L1 fibroblasts were obtained from the National Center for Cell Science, Pune, India. The cells were cultured in Dulbecco’s modified Eagle medium (DMEM) containing 10% fetal calf serum (FCS), 100 Uml⁻¹ penicillin and 100gml⁻¹ streptomycin (standard medium). The cells were inoculated into six multidishe at a density of 9×10³ cells/well with medium and then maintained at 37 °C under a humidified 5% CO2 atmosphere. The number of cells was determined with a hemacytometer, using trypsin-dissociated cells. Two days after confluence (day 0), cells were fed with a differentiation medium (fresh standard medium containing 0.5mM 3-isobutyl-1-methylxanthine, 0.2 M dexamethasone and 6gml⁻¹ insulin). After 2 days, the medium was removed and then replaced with a post-differentiation medium (fresh standard medium containing 6 gml⁻¹ insulin) and then the medium was changed every other day. At day 14, more than 90% of cells had accumulated lipid droplets, and therefore were considered to be adipocytes.

**Animals**

Twenty four, C57BL/6J female mice (the twelfth day of pregnancy) were obtained from Jay Research Foundation, Vapi, India. Animals were maintained in a temperature-controlled room (22±1 °C, relative humidity 55±3%) with an automatic regimen of light darkness of 12 h×12 h. Food (Pranav Agro, Baroda, India) and water were supplied ad libitum. The handling of the laboratory animals was performed according to CPCSEA guideline, India and
all the studies were approved by the Institutional animal ethical committee (IAEC), (Ref: IAEC/2012/03) SSR College of Pharmacy, Silvassa, India.

High-Fat Diet-Induced Obesity

After 14 days of acclimation, female mice were randomly divided into 4 groups, each group having 6 animals: Group I (lean 6 mice) had free access to standard pelleted chow which provided 64.41 % of energy as carbohydrates, 22.46 % as protein, and 12.61 % as fat. Remaining three group (18 mice) were fed with a high fat diet providing 58.23 % of energy as fat, 15.69 % as protein and 26.08 % as carbohydrates throughout the experiment. High fat diets were formulated as a modification of Surwit et al.\textsuperscript{[18]} and West et al.\textsuperscript{[19]} Refer Table 1. Group II were fed with High-fat diet during the entire study (8 week) and administered 2 ml/kg of ISS, p.o/day, for 6 week. Group III were fed with High-fat diet during the entire study (8 week) and administered 286 mg/kg/day (low dose) of the \textit{Pinus sylvestris} bark extract, p.o/day, for 6 week. Whereas group IV were fed with High-fat diet during the entire study (8 week) and administered 667 mg/kg/day of (high dose) the \textit{Pinus sylvestris} bark extract, p.o/day, for 6 week. PS extract is soluble in ISS so it was used as media to dissolve extract and animals were administrated with 286 mg/kg/day and 667 mg/kg/day of PS-extract by oral gavages (using a 22-gauge, 25mm stainless steel feeding needle) for 6 weeks. The food intake, water intake and body weight were measured weekly. The mean daily food intake per mouse calculated in unit of g/day/mouse where mean daily water intake per mouse calculated in unit of ml/day/mouse.

Table 1: Composition of the experimental diets\textsuperscript{[18-21]}

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Regular diet (g/kg diet)</th>
<th>High fat diet (g/kg diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>3.0</td>
<td>3</td>
</tr>
<tr>
<td>Corn Starch</td>
<td>500</td>
<td>260</td>
</tr>
<tr>
<td>Sucrose</td>
<td>150</td>
<td>110</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Corn oil</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>Beef tallow</td>
<td>-</td>
<td>330</td>
</tr>
<tr>
<td>Mineral mixture \textsuperscript{b}</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mixture \textsuperscript{a}</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>l-cystein</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Vitamin Mix for AIN- 76A, \textsuperscript{b}Mineral Mix for AIN- 76A
Biochemical parameters
At the end of the experimental protocol, the plasma concentrations of glucose, total cholesterol and Triglyceride were determined using biochemical kits, glucose by GOD-POD, end point assay, Cholesterol by one step method and Triglycerides by GPO-PAP, end point method, according to manufacturer’s instructions (Span diagnostics, India). Plasma insulin, adiponectin and leptin were assessed by enzyme linked immunoassay kit i.e. Mouse INS (Insulin) ELISA Kit, Mouse ADP/Acrp30 (Adiponectin) ELISA Kit and Mouse LEP (Leptin) ELISA Kit respectively, according to manufacturer’s instructions (Elabscience Biotechnology Co. Ltd.)

Organ weight
At the end of the experimental protocol, the mice were sacrificed by cervical dislocation. During the sacrifice, various organs were removed, collected and weighed; notably, liver, white adipose tissue (WAT; periovarian, perirenal, and mesenteric fat pad), and subscapular brown adipose tissue (BAT). Adiposity index, a quantitative measure of total fat mass was also calculated using the previously determined equation.\[22\]

\[\text{Adiposity Index (\%) = \left(\frac{fat\ pad}{Body\ weight}\right) \times 100}\]

Pancreatic Lipase Inhibition Assay
Porcine pancreatic lipase (PPL, type II) activity of various concentrations of PS-extract (0-250 μg/ml) was measured using p-nitrophenyl butyrate (p-NPB) as a substrate. The method used for measuring the pancreatic lipase activity was modified from that previously described by Zheng, et al.\[23\] The activity of the negative control was also examined with and without an inhibitor. The inhibitory activity (I) was calculated according to the following formula.

\[\text{Inhibition (\%) = } \left\{1 - \frac{(Abs6 - Abs5)}{(Abs8 - Abs7) \times 100}\right\},\]

Where Abs5 is the absorbance of incubated solution containing PS, substrate, and lipase; Abs6 is the absorbance of incubated solution containing PS and substrate; Abs7 is the absorbance of incubated solution containing substrate and lipase; Abs8 is the absorbance of incubated solution containing substrate.

Cytotoxicity assay using 3T3-L1 cells
The 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) colorimetric assay\[24\] was followed with little modifications.\[25-27\] 3T3-L1 cells were seeded in 96 well
plates (10^4 cells/well) separately and exposed to various concentrations of PS-extract (50-500 μg/ml). After 24, 48 and 72 h of incubation, 10 μl of MTT was added (5 mg/ml) into each well of 96 well plates and incubated for 4 h. The precipitated formazan salt was dissolved in 100 μl of isopropanol. The plate samples were read at 570 nm with a microtiter plate reader. Cell survival was expressed as percentage of viable cells of treated samples compared to control samples. Each experiment was performed in triplicate.

**Statistical analysis**

The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey comparison test. The values are expressed as mean±SD and P<0.05 was considered significant.

**RESULTS**

**Preliminary phytochemical analysis of PS- extract.**

The percentage yield of PS extract was found 8.45 %. Total Phenolic Content (Gallic Acid Equivalent) and Total Flavonoid Content (Quercetin Equivalent) of PS was found to be 52.03±5.1 gm % and 47.32±2.6 gm % respectively.

**Acute toxicity study**

There was no mortality observed with PS extract up to 2000 mg/kg body weight of animals and other symptoms observed during the first 4 h includes decreased motility, sedation, abdominal twisting and increased urination. So 1/7th and 1/3rd of 2000 mg/kg i.e., 286 mg/kg and 667 mg/kg of doses were selected for study anti-obesity effect.

**Food and water intake, Body weight, and adipose tissues**

The calorie intake values in cal/day/mouse from 0 to 8 weeks of HFD diet supplement are represented in Fig 1. At all points, the food intake was significantly higher and water intake was lower in HFD treated group than in the normal mice. In HFD- induced control mice water intake was 6.5±2.1 ml/day/ mouse whereas normal control mice 9.3±3.2 ml/day/ mouse. However, the both doses of PS supplementation tended to reduce calorie intake as compared with the high-fat-fed control group but only after 7 weeks of PS (667 mg/kg) supplementation significantly reduced calorie intake (p < 0.01) as compared with the HFD control group. (Fig. 1).
The body weight of the HFD treated group rats was increased rapidly after 18 days, and becoming significantly higher than the control group after 42 days HFD diet supplement, represented in Fig 2 (p<0.001). After 8 weeks, the final body weight and the weight gain in the high-fat diet induced obese mice were 46.67±2.07 g and 20.31±1.74 g, respectively, which were significantly higher than those in normal diet-fed mice; The PS (667 and 286 mg/kg/d) supplements significantly reduced body weight gain compared to the high-fat control group by 16.95% and 20.65%, respectively (Table 2). At the end of the experiment, liver weights of the HFD + PS-LD group were 8.21 % less than the HFD control group (p > 0.05). In response to PS-HD, high-fat diet induced increases in adipose tissue weights were significantly decreased by 19.61 % (p<0.05). Thus, PS inhibits high-fat diet-induced adipose tissue mass in mice. Weights of periovarian, perirenal and mesenteric adipose tissues were decreased by 19.61 %, 30.85 % and 16.06 % in HFD + PS-HD mice group (p> 0.05), respectively, compared with those in the HFD control group (Fig. 5.1C); Coherent with the decreases in WAT, BAT was also decreased in the both HFD + PS-HD mice group and HFD + PS-LD group compared with the HFD control group. The adiposity index was markedly increased in HFD treated groups as compare to control group (Table 2). After 8 week PS-HD treatment the adiposity index of HFD- induced obese mice flattened significantly (p<0.05) compared with untreated HFD- induced obese mice.

![Figure 1: Effects of PS on Energy intakes (cal/day/mouse) in mice fed high-fat (HF) and low-fat (LF) diets for 8 weeks.](image-url)

Plotted values are the mean ± SD with n=06 mice per group.
Figure 2: Body weights in mice fed with either high-fat (HF) and low fat (control) diets or HFD in combination with PS (286 and 667 mg/kg) for 8 weeks.

Plotted values are the mean ± SD with n=06 mice per group. *** p<0.001 extremely significant, ** p<0.01 very significant, * p<0.05 significant, (no marking) p>0.05, no significant, PS treatment significant compared to HFD control, whereas HFD control significant compared to normal control.

Table 2: Effect of PS on body weight, weight gain, food intake, visceral fat weight, BAT and adiposity index level in mice fed a high-fat and low-fat diet for 8 weeks.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Control</th>
<th>HFD control</th>
<th>HFD + PS-LD</th>
<th>HFD + PS-HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Body weight (g)</td>
<td>27.23±1.70</td>
<td>26.37±2.04</td>
<td>28.17±1.08</td>
<td>27.85±2.45</td>
</tr>
<tr>
<td>Final Body weight (g)</td>
<td>37.35±1.70</td>
<td>46.67±2.07***</td>
<td>45.04±3.19</td>
<td>43.96±2.76</td>
</tr>
<tr>
<td>Body weight gain (g)</td>
<td>10.12±1.50</td>
<td>20.31±1.74***</td>
<td>16.87±3.45***</td>
<td>16.11±1.19***</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>1.51±0.17</td>
<td>1.38±0.14ns</td>
<td>1.42±0.13ns</td>
<td>1.40±0.12ns</td>
</tr>
<tr>
<td>Periovarian WAT (g)</td>
<td>0.61±0.02</td>
<td>1.15±0.15***</td>
<td>1.03±0.20ns</td>
<td>0.95±0.18*</td>
</tr>
<tr>
<td>Perirenal WAT (g)</td>
<td>0.23±0.03</td>
<td>0.65±0.10***</td>
<td>0.52±0.14ns</td>
<td>0.45±0.16*</td>
</tr>
<tr>
<td>Mesenteric WAT (g)</td>
<td>0.40±0.04</td>
<td>1.25±0.21***</td>
<td>1.09±0.25ns</td>
<td>1.05±0.26ns</td>
</tr>
<tr>
<td>Whole WAT (g)</td>
<td>1.23±0.09</td>
<td>3.05±0.45***</td>
<td>2.64±0.58ns</td>
<td>2.45±0.55*</td>
</tr>
<tr>
<td>BAT</td>
<td>0.11±0.03</td>
<td>0.54±0.15***</td>
<td>0.43±0.18ns</td>
<td>0.37±0.19ns</td>
</tr>
<tr>
<td>Adiposity index</td>
<td>3.30±0.10</td>
<td>6.54±0.71***</td>
<td>5.85±0.94ns</td>
<td>5.58±0.88*</td>
</tr>
</tbody>
</table>

Plotted values are the mean ± SD with n=06 mice per group. *** p<0.001 extremely significant, ** p<0.01 very significant, * p<0.05 significant, (no marking) p>0.05, no significant, PS treatment significant compared to HFD control, whereas HFD control significant compared to normal control, Negative value (-) indicate a reduction in body weight.
Plasma lipid Profile

As shown in Table 3, 8 weeks fed a high fat diet in mice, the plasma levels of glucose, triglyceride and total cholesterol were higher in the HFD-control group than those in the normal group (p < 0.001), which indicated that High fat diet was successful inducing hyperlipidemia in mice. The increased plasma levels of glucose were significantly suppressed, by PS-HD treatment in HFD-induced mice and it was 14.96 % reduction as compared to HFD control group respectively. Similar to plasma glucose concentration, the increased plasma levels of triglyceride and total cholesterol were significantly suppressed, by PS-HD treatment in HFD induced mice.

Table 3: Effect of PS on biochemistry of mice fed a high-fat and low-fat diet for 8 weeks

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HFD</th>
<th>HFD+PS-LD</th>
<th>HFD+PS-HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>79.70±6.55</td>
<td>116.83±9.78***</td>
<td>106.19±13.00ns</td>
<td>99.35±12.64</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>77.77±8.67</td>
<td>121.23±13.30**</td>
<td>109.66±9.43</td>
<td>93.57±11.29***</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>64.11±9.39</td>
<td>107.08±7.19***</td>
<td>91.01±11.39</td>
<td>84.54±10.38***</td>
</tr>
<tr>
<td>Insulin (ng/mL)</td>
<td>4.17±0.82</td>
<td>20.17±1.62***</td>
<td>15.05±1.84**</td>
<td>10.28±1.30**</td>
</tr>
<tr>
<td>Adeponectin (ng/mL)</td>
<td>8.34±0.94</td>
<td>3.07±0.47</td>
<td>4.52±1.17ns</td>
<td>5.74±0.96**</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>8.74±1.05</td>
<td>18.23±1.43***</td>
<td>15.88±1.02**</td>
<td>12.83±1.15***</td>
</tr>
</tbody>
</table>

Plotted values are the mean ± SD with n=06 mice per group., ***p<0.001 extremely significant, **p<0.01 very significant, *p<0.05 significant, ns,p>0.05, no significant, PS treatment significant compared to HFD control, whereas HFD control significant compared to normal control.

Figure 3: Effect of PS on percentage change in plasma Insulin, Adeponectin and Leptin level as compare with untreated mice fed a high-fat and low-fat diet for 8 week.

Plotted values are the mean ± SD with n=06 mice per group.
Plasma leptin, insulin and adiponectin levels
The high-fat control diet cause to increase the plasma leptin and insulin levels significantly compared to the normal diet fed mice, while it decreased the plasma adiponectin level. However, PS-HD treatment significantly reduced the plasma insulin and leptin levels in HFD induced obese mice by about 50.96% and 70.40 % respectively (Fig 3). The HFD-induced decreases in the plasma adiponectin levels of the mice were also reversed by administration of.

Porcine pancreatic lipase (PPL) inhibitory activities of PS.
Crude extracts of Pinus sylvestris PPL inhibitory effects at different concentrations, and a dose-response curve was obtained, as shown in Fig 4. The significant inhibition of PPL was observed up 28% with Pinus sylvestris bark. Treatment with Orlistat (at final concentration 250 µg/ml) as a positive control, a well-known anti-lipase agent, significantly inhibited the PPL activity up to 52.61 %.

![Figure 4: Porcine pancreatic lipase (PPL) inhibitory activities of PS. Orlistat was used as a positive control. Experiments have been performed in triplicate.](image)

Plotted values are the mean ± SD with n=3 Trial

Effect of PS on inhibition of proliferation in 3T3-L1 preadipocytes:
As shown in Fig 5, PS inhibited the growth of 3T3-L1 preadipocytes in a time- and concentration-dependent manner and revealed antiproliferative activity under all treatment conditions. The antiproliferative activity of PS after 72 h was stronger than that after 24 and 48 h, reductions of 28.8 % and 27.0 % at 72 h for 500 and 1000 µg/ml PS respectively, were noted, as compared with the control levels after 72 h.
Figure 5: Effect of PS on the inhibition of cell proliferation in 3T3-L1 preadipocytes

Plotted values are the mean ± SD with n=3 Trial

DISCUSSION

Several preparations of plants used in traditional medicine to cut down obesity could be utilized to cancel the clinical side effects of the current chemically formulated anti-obesity agents. No toxic effects were observed when the normal healthy mice were administered with the extracts (2000 mg/kg/day). These observations showed that the doses used for the study were safe. We describe here a model of diet-induced obesity in mice that is reproducible over several experiments, well controlled and shares many features with human obesity. Reviews of dietary obesity describe potential mechanisms of body weight and food intake regulation involving the central nervous system, i.e. the hypothalamus, neuropeptides i. e. ghrelin and neuropeptide Y and hormones i.e. insulin and leptin.

Mice fed a high fat diet shows distinctive visceral adiposity, dyslipidemia, hyperglycemia, hyperinsulinemia and hepatic steatosis, that are typically associated with human obesity. Results presented in this study show that high-fat diet fed to mice increases body weight gain, liver weight, WAT weight, and IBAT weight as compare to normal mice keeping constant the daily food or energy intake. Plasma level of glucose, total cholesterol and triglyceride in HFD- induced obese mice significantly and dose dependent manner increase as compared to normal control mice. We observed that mice fed a high-fat diet supplemented with PS-extract for 8 weeks significantly decreased the levels of serum triglycerides and total cholesterol compared to mice fed a high-fat diet alone. This data indicating that above plants extract efficiently regulates triglyceride and cholesterol metabolism in obese mice. Thus, PS may be useful for treating patients with hypercholesterolemia and hypertriglyceridemia.
In parallel with effect on serum triglycerides, mice fed a high-fat diet, supplemented PS had significantly lower adipose tissue mass and body weight gain than mice fed a high fat diet alone. This correlation with reports shows that the lipids in adipose tissue are largely derived from circulating triglycerides, especially during high-fat diet and so the reductions in serum triglycerides also lead to decreased adipose tissue.\[^{32}\] In addition, PS not significantly affects the food intake of high-fat diet-fed obese mice. These observations together indicate that PS significantly reduces adipose tissue mass and body weight gain by increasing fat catabolism in the liver, but not by influencing food intake.

Obesity is associated with elevated leptin production in the blood.\[^{33}\] Leptin exerts negative feedback effects on energy intake, but loses its ability both, to inhibit energy intake and stimulate energy expenditure in conditions of obesity. Similarly, our result shows that an obese control was higher levels of plasma leptin compared with control. Several authors have reported that aging\[^{34}\] or the consumption of a HFD\[^{35}\] results in the development of leptin resistance in rodents, measured as a failure of leptin either to inhibit food intake or to induce weight loss. Increased circulating leptin, a marker of leptin resistance, is common in obesity and independent associates with insulin resistance\[^{36}\] and CVD\[^{37}\] in humans. In our study, the Serum leptin in model group was raised during the induction of HFD. After the treatment of PS-plant extract, the serum leptin is significantly decreased as compared with model group. It suggested that the plant extract was effective to leptin resistance induced by HFD in mice. Several studies have pointed out that the most common form of insulin resistance is associated with an increased accumulation visceral fat.\[^{38}\] In addition to this also reported, insulin resistance or insulin deficiency is associated with hypercholesterolemia and hypertriglyceredemia. Insulin resistance may be responsible for dyslipidemia, because insulin has an inhibitory action on HMG-CoA reductase, a key rate limiting enzyme responsible for the metabolism of cholesterol rich LDL particles.\[^{39}\] Prevention of hyperinsulinemia may improve metabolic abnormalities that occur in the liver as a consequence of obesity.\[^{40}\] It was also observed that both the body weight and adipose tissue weight were positively correlated with plasma leptin and insulin levels respectively. The plasma leptin and insulin levels were significantly higher in the HFD-induced mice group than in the normal mice group; however, these hormone levels were significantly lowered by PS treatment. The reduction glucose, cholesterol and triglyceride level in the condition of obesity associated with insulin resistance indicates that the plant extract improves insulin sensitivity. Plasma adiponectin levels have been reported to be significantly reduced in obese/diabetic mice and humans\[^{41}\] and to be...
decreased in patients with cardiovascular disease[^42], hypertension[^43], or metabolic syndrome.[^44] Although PS significantly decreased elevated plasma adiponectin concentration compared to the HFD-induced obese mice group.

Pancreatic lipase is the key enzyme for lipid absorption that hydrolysis triacylglycerol in the GIT. Pancreatic lipase inhibitor which help in limit intestinal fat absorption at initial stage have been proved as useful medications for the treatment of hyperlipidaemia and a great promise as anti-obesity agents.[^45] Pancreatic lipase inhibition is one of the most widely studied mechanisms used to determine the potential efficiency of natural products as anti-obesity drugs.[^46] The significant inhibition of PPL was observed up 28% with *Pinus sylvestris* bark. The results of the MTT assay clearly indicated that PS showed the inhibition of cell proliferation on 3T3-L1 preadipocytes. Many studies in different cell lines, animal models, and human epidemiological trials have shown the potential of dietary polyphenols as antiproliferation agents.[^47] The inhibition of proliferation and induction of apoptosis are regulated by a network of signaling pathways and transcription factors, which are possible targets for a rational tumor therapy.[^48-49]

The mechanisms by which PS causes a reduction in body weight gain are not yet clear. But an understandable explanation for these effects of PS bark is due to very rich phytoconstituents of it such as flavonoid[^7], essential oil[^8] dipentene, pinene, sylvestrene, cadinene, bomyl acetate[^9] phenolic acid (dihydroxybenzoic acid), Pinolenic acid and sterol (sitosterol).[^10] It has been reported that A 3, 5-dihydroxybenzoic acid[^50] and Pinolenic acid[^11] or their derivative compound having anti-obesity activity. In the present study, the quantification of total phenolic and flavonoids content in PS was performed. However, our results did not permit a determination of whether these constitutes were responsible for the anti-obesity effect of PS.

**CONCLUSION**

In conclusion, *Pinus sylvestris* bark inhibited proliferation of 3T3-L1 adipocytes, reduced adipose tissue mass, hyperlipidemia, improve insulin and leptin sensitivity and reduced adiponectin levels, in obese rats fed HFD. Therefore, *Pinus sylvestris* bark extract may be considered for use in therapy to control obesity. However, further work is needed to elucidate the possible mechanisms by which bark of *Pinus sylvestris* extract intervenes.
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