INTRODUCTION

Obesity possess a negative impact on the health of an individual, due to fat accumulation in the body. Globally 400 million people are obese with body mass index above 30. Obesity is prevalent not only in developed countries but also in developing countries. Obesity predisposes to diseases such as hypertension, dyslipidemia, atherosclerosis, type 2 diabetes mellitus, and degenerative joint disorders and cancer. Apart from an imbalance between the energy consumption and its utilization, hormones play a prominent role in the occurrence of obesity. Diverse hormones such as leptin, insulin, sex hormones and growth hormone greatly influence the appetite, metabolism, and body fat distribution and storage.

Obesity predisposes to diseases such as hypertension, dyslipidemia, atherosclerosis, type 2 diabetes mellitus, and degenerative joint disorders and cancer. Apart from an imbalance between the energy consumption and its utilization, hormones play a prominent role in the occurrence of obesity. Diverse hormones such as leptin, insulin, sex hormones and growth hormone greatly influence the appetite, metabolism, and body fat distribution and storage.

INTRODUCTION

Obesity possess a negative impact on the health of an individual, due to fat accumulation in the body. Globally 400 million people are obese with body mass index above 30. Obesity is prevalent not only in developed countries but also in developing countries. Obesity predisposes to diseases such as hypertension, dyslipidemia, atherosclerosis, type 2 diabetes mellitus, and degenerative joint disorders and cancer. Apart from an imbalance between the energy consumption and its utilization, hormones play a prominent role in the occurrence of obesity. Diverse hormones such as leptin, insulin, sex hormones and growth hormone greatly influence the appetite, metabolism, and body fat distribution and storage.

ABSTRACT

Objective: The prevalence of obesity-associated comorbidities, enhance the risk for predominant health problems such as diabetes, osteoarthritis, and certain cancers. Natural product based intervention has been one of the crucial strategies for management of obesity ailments. The study was intended to investigate the anti-obesity activity of ethanolic extract of *Vitis vinifera* in butter and progesterone induced obese rats.

Materials and Methods: Leaves of *V. vinifera* were subjected to Soxhlet extraction with alcohol. Obtained alcoholic extract was subjected to phytochemical screening and acute toxicity studies. In progesterone induced obesity model progesterone (10 mg/kg), standard drug, sibutramine (10 mg/kg), and *V. vinifera* (200 mg/kg, 400 mg/kg) were administered for 28 days. On the 29th day, blood samples were isolated for assessing biochemical parameters such as glucose, triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very LDL (VLDL), serum glutamate oxaloacetate transaminase (SGOT), and serum glutamate pyruvate transaminase (SGPT). Liver was isolated for histopathological studies. In Butter induced obesity model, 400 mg/kg butter, standard drug, atorvastatin (10 mg/kg), and *V. vinifera* (250 mg/kg and 500 mg/kg) were administered for 21 days. After 21 days blood was withdrawn for estimating LDL, VLDL, HDL, cholesterol, and TG.

Results: Phytochemical screening revealed the presence of glycosides, alkaloids, flavonoids, steroids, and tannins. By acute toxicity studies, *V. vinifera* was found to be safe up to 1500 mg/kg. In progesterone induced obesity model, sibutramine and *V. vinifera* significantly (*P* < 0.01) decreased glucose, TC, TG, LDL, VLDL, SGOT, and SGPT along with an increase in HDL. In butter induced obesity model, atorvastatin and *V. vinifera* significantly (*P* < 0.01) decreased glucose, TC, TG, LDL, and VLDL level long with significant increase in HDL. Histopathological studies revealed reduced necrosis and fatty changes of liver in sibutramine (10 mg/kg), and *V. vinifera* (200 mg/kg and 400 mg/kg) treated group compared to progesterone-treated group.

Conclusion: Results of the study reveal anti-obesity activity of *V. vinifera*.

KEY WORDS: Butter, Lovostatin, Obesity, Progesterone, Sibutramine, *Vitis vinifera*
exerts neuroprotective and neurogenic action as well as regulates the release of various neurotransmitters. Progesterone induces hyperphagia and thereby increases fat deposition and weight gain by interfering with carbohydrate and lipid metabolism. Progesterone induced obesity is one of the well-established models for screening anti-obesity agents. However, herbal formulations are gaining major limelight due to synergistic action and lesser side effects when compared to synthetic moieties.

*Vitis vinifera* belonging to family Vitaceae is an Asian perennial woody vine. Leaves have been employed for treating diarrhea, hemostatic, attention deficit hyperactivity disorder, chronic fatigue syndrome, heavy menstrual bleeding, uterine bleeding, canker sores, mild laxative, and external wound healing and to lance abscesses. Grapes, seeds, and leaves have been used for preventing heart and blood vessels diseases, varicose veins, hemorrhoids, “hardening of the arteries” (atherosclerosis), high blood pressure, swelling after injury or surgery, heart attack, and stroke. Leaf-based medicines were employed traditionally for diarrhea, hepatitis, and stomach aches. Scientifically, leaves were reported to possess antidiabetic and antioxidant activities, antibacterial activity, antileishmanial activity, and antioxidant activity. The plant is rich in phytoconstituents such as flavonoids, tannins, polyphenolics, anthocyanins, catechins, micronutrients, and vitamins. The present study was performed to assess the anti-obesity activity and to prove its claim against folklore practice.

**MATERIALS AND METHODS**

**Procurement of Plant**

The plant materials (leaves) were collected from Talakona forest, Tirupati. Authentication was done by Dr. S. Madhava Chetty, Assoc. Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, and the voucher (specimen) was preserved with a specimen no.1165 [Figure 1].

**Animals**

Healthy adult Wistar albino rats (150–200 g) and Swiss albino mice (30–40 g) were procured from Mahaveer Enterprises, Hyderabad, India. Experiments were conducted according to the Institutional Animal Ethical Committee approval with CPCSEA Regd.NO 1684/PO/Re/S/13/CPCSEA. Rats were housed under natural photoperiodic and standard atmospheric conditions with access to food and water *ad libitum*.

**Extraction Process**

Leaves of *V. vinifera* were shade dried, powdered and passed through sieve number 20. Powdered leaves were extracted by the soxhleation process. Obtained extracts were concentrated under reduced pressure at 40°C. The percentage yield was 14%.

**Preliminary Phytochemical Analysis**

The ethanolic extract of *V. vinifera* was subjected to preliminary phytochemical evaluation for the presence of phytoconstituents.

**Toxicity Studies**

Ethanolic extract of *V. vinifera* did not produce any toxic symptoms or mortality up to the dose level of 500 mg/kg body weight in mice, 1500 mg/kg body weight in rats, and hence, the extract was considered to be safe and non-toxic for further pharmacological screening.

**Chemicals**

- Butter - Amul butter, Hyderabad.
- Progesterone - LUPIGET (Lupin Pharma).
- Atorvastatin - gift sample by Moral Labs, Chennai.
- Serum triglyceride (TG) kit - AngStrom Biotech private Ltd., Vadodara.

**Figure 1:** *Vitis vinifera* leaves

**Figure 2:** (a) Group I: Normal control group, (b) Group II: Progesterone (10 mg/kg), (c) Group III: *Vitis vinifera* (250 mg/kg), (d) Group IV: *Vitis vinifera* (500 mg/kg), (e) Group V: Sibutramine (10 mg/kg)
Anti-obesity Activity

**Progesterone induced model**

Rats were segregated into groups containing six each. Group I received vehicle (saline), served as control; Group II received progesterone (10 mg/kg, S.C); Group III received *V. vinifera* (200 mg/kg; p.o)+ progesterone (10 mg/kg, S.C), Group IV received *V. vinifera* (400 mg/kg; p.o)+ progesterone (10 mg/kg, S.C); and Group V received, standard drug, sibutramine (10 mg/kg; p.o)+ progesterone (10 mg/kg, S.C). *V. vinifera* was given 30 min before progesterone administration. Progesterone is solubilized in arachis oil and given subcutaneously in the dorsal neck region. Treatment was given for 28 days. On the 29th day, blood samples were collected from retro-orbital puncture. Serum was isolated, centrifuged and employed for analyzing lipid profiles.

**Histopathological Examinations**

At the end of the experiment on the 29th day, rats were sacrificed by cervical dislocation; liver was isolated and stored in 10% formalin solution. The sections of liver were stained with hematoxylin and eosin and observed under light microscope (×100).

Butter Induced Model

Rats were divided into groups containing six each. Group I received vehicle (saline), served as control; Group II received butter (400 mg/kg, p.o); Group III received *V. vinifera* (250 mg/kg; p.o)+ butter (400 mg/kg, p.o), Group IV received *V. vinifera* (400 mg/kg; p.o)+ butter (400 mg/kg, p.o); and Group V received, standard drug, atorvastatin (10 mg/kg; p.o)+ butter (400 mg/kg, p.o). Treatment was given for 20 days. On the 21st day, blood samples were collected from retro-orbital puncture. Serum was

### Table 1: Phytochemical screening of *V. vinifera* leaves

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
</tbody>
</table>

### Table 2: Effect of *V. vinifera* on glucose, lipid levels and liver enzymes in progesterone-induced obesity model (mean±SEM)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg)</th>
<th>Glucose (mg/dl)</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
<th>SGOT (mg/dl)</th>
<th>SGPT (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control (saline)</td>
<td>139.53±14.01</td>
<td>101.15±5.67</td>
<td>80.90±8.15</td>
<td>27.06±0.550</td>
<td>59.91±6.10</td>
<td>16.18±1.44</td>
<td>129.30±5.30</td>
<td>60.83±6.98</td>
</tr>
<tr>
<td>II</td>
<td>Progesterone (10)</td>
<td>190.41±11.11**</td>
<td>140.16±6.27</td>
<td>111.43±9.30</td>
<td>23.33±1.024</td>
<td>73.10±4.10</td>
<td>22.28±1.64</td>
<td>160.60±5.52</td>
<td>68.08±5.28</td>
</tr>
<tr>
<td>III</td>
<td><em>V. vinifera</em> (200)</td>
<td>147.35±9.67**</td>
<td>100.16±6.27</td>
<td>90.80±7.10</td>
<td>28.90±1.040</td>
<td>64.00±4.10</td>
<td>18.16±2.22</td>
<td>126.1±10.41</td>
<td>70.00±9.51</td>
</tr>
<tr>
<td>IV</td>
<td><em>V. vinifera</em> (400)</td>
<td>116.2±5.65**</td>
<td>110.01±5.67</td>
<td>90.80±7.10</td>
<td>28.90±1.040</td>
<td>64.00±4.10</td>
<td>18.16±2.22</td>
<td>126.1±10.41</td>
<td>70.00±9.51</td>
</tr>
<tr>
<td>V</td>
<td>Sibutramine (10)</td>
<td>140.32±6.62**</td>
<td>85.7±4.73</td>
<td>79.39±6.89</td>
<td>33.97±0.842</td>
<td>35.85±2.41</td>
<td>15.87±2.18</td>
<td>129.2±11.19</td>
<td>65.98±7.58</td>
</tr>
</tbody>
</table>

n=5; Group II is compared with Group I. Groups III-VI were compared with Group II. *P*<0.05, **P*<0.01, ***P*<0.001. *V. vinifera: Vitis vinifera*
isolated, centrifuged and employed for analyzing lipid profiles.

Biochemical Estimations

On the 29th day in progesterone-induced model and on the 21st day in the butter induced model, blood was collected by retro-oral sinus puncture. Centrifugation was done at 3000 rpm for 15 min. Isolated serum was employed for analyzing blood glucose,[30] TC,[30] high-density lipoprotein (HDL),[30] TG,[31] SGOT, and SGPT[32] were estimated using commercial diagnostic kits. Low-density lipoprotein, and very LDL (VLDL) levels were calculated according to Friedwald’s formula.[33]

\[
\text{LDL} = \frac{\text{TC} - (\text{HDL} + \frac{\text{TG}}{5})}{5}
\]

\[
\text{VLDL} = \frac{\text{TG}}{5}
\]

Statistical Analysis

Results were expressed as mean ± SEM. Statistical significance was calculated by GraphPad InStat version 3 followed by Dunnet’s test. \( P < 0.05 \) was considered to be statistically significant.

RESULTS

Phytochemical screening revealed the presence of alkaloids, glycosides, flavonoids, steroids, and tannins [Table 1]. Acute toxicity studies indicated that \( V. \) \( vinifera \) was non-toxic up to 1500 mg/kg. Subcutaneous administration of progesterone for 28 days significantly increased glucose \(( P < 0.01)\), cholesterol \(( P < 0.01)\), TG \(( P < 0.01)\), LDL \(( P < 0.01)\), VLDL \(( P < 0.01)\), SGOT \(( P < 0.01)\), SGPT \(( P < 0.01)\), and decreased HDL levels when compared to control rats. Treatment with \( V. \) \( vinifera \) (200 mg/kg and 400 mg/kg) significantly decreased the elevated glucose \(( P < 0.01)\), cholesterol \(( P < 0.01)\), TG \(( P < 0.01)\), LDL \(( P < 0.01)\), VLDL \(( P < 0.01)\), SGOT \(( P < 0.01)\), SGPT \(( P < 0.01)\), and increased HDL levels \(( P < 0.01)\) when compared to progesterone treated rats [Table 2]. Administration of butter for 21 days significantly increased TG \(( P < 0.01)\), cholesterol \(( P < 0.01)\), LDL \(( P < 0.01)\), and decreased HDL \(( P < 0.01)\) levels when compared to control group. \( V. \) \( vinifera \) (200 mg/kg and 400 mg/kg) significantly attenuated the elevated levels of TG \(( P < 0.01)\), cholesterol \(( P < 0.01)\), LDL \(( P < 0.01)\), VLDL \(( P < 0.01)\), and increased the attenuated HDL levels \(( P < 0.01)\) [Table 3].

Histopathology of Liver

As manifested in the Figure 2, the hepatocytes of normal group are found to be normal. There was absence of factors like necrosis, fat globules. The central vein is evident in the image. In progesterone induced group the presence of fatty change was observed in the liver cells indicating progesterone is successfully responsible for the induction of the obesity and occurrence of fat globules. \( V. \) \( vinifera \) (250 mg/kg) brought changes like focal necrosis and slight hepatotoxicity which proves that there is action of the drug and absence of fat globules. \( V. \) \( vinifera \) (500 mg/kg) there is significant and increased focal necrosis and total absence of fat globules and this extract shows perfect decrease in the fat cells which were induced by progesterone. Sibutramine (10 mg/kg) showed mild necrosis.

DISCUSSION

Progesterone administration caused impairment in fat and carbohydrate metabolism is reflected in the biochemical parameters such as blood glucose and lipid profile. Elevated progesterone level leads to gestational diabetes in pregnancy due to hyperphagia. Thus, in the current study progesterone-treated group evidenced significantly elevated blood glucose level that has been completely reversed with \( V. \) \( vinifera \) 200 mg/kg and 400 mg/kg.[34,35] Progesterone modulated various biochemical parameters by stimulation of lipoprotein lipase, that hydrolyse dietary fat leading to enhancement of fat storage in the body, and thereby enhanced lipid profile.[36] \( V. \) \( vinifera \) decreased lipid profiles by inhibiting lipoprotein lipase indicating anti-obesity activity. Elevated SGOT, SGPT indicate hepatic abnormality or injury.[37] Treatment with \( V. \) \( vinifera \) 200 mg/kg and 400 mg/kg significantly attenuated SGOT, SGPT levels in contrast to progesterone and butter induced group indicating hepatoprotective activity. The histopathological examination evidences the harmful effects of the drug on vital organs.[38] The section of liver in progesterone-treated group indicated distorted structures of functional unit as mild congestion and focal necrosis of hepatocytes. However, the hepatic architecture was restored with \( V. \) \( vinifera \) (200 mg/kg and 400 mg/kg) treatment.

---

Table 3: Effect of \( V. \) \( vinifera \) on lipid levels in butter induced obesity model (mean±SEM)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control (saline)</td>
<td>158±7.58</td>
<td>114±6.47</td>
<td>50±3.58</td>
<td>85±2±2.22</td>
<td>22.8±2.47</td>
</tr>
<tr>
<td>II</td>
<td>Butter (400 mg/kg)</td>
<td>242.40±12.48**</td>
<td>370.0±13.42**</td>
<td>35.0±1.50**</td>
<td>133.4±3.21**</td>
<td>74.4±4.58**</td>
</tr>
<tr>
<td>III</td>
<td>( V. ) ( vinifera ) (250)</td>
<td>202.90±8.58**</td>
<td>185.0±9.34**</td>
<td>44±1.20*</td>
<td>121.9±2.11*</td>
<td>37±3.51**</td>
</tr>
<tr>
<td>IV</td>
<td>( V. ) ( vinifera ) (500)</td>
<td>180.0±7.48**</td>
<td>168.50±10.17**</td>
<td>46±1.17*</td>
<td>100.3±3.12**</td>
<td>33.7±4.55**</td>
</tr>
<tr>
<td>V</td>
<td>Atorvastatin (10)</td>
<td>159.20±9.92**</td>
<td>164.52±9.51**</td>
<td>48.58±3.31**</td>
<td>77.71±3.05**</td>
<td>32.90±4.75**</td>
</tr>
</tbody>
</table>

\( n=5; \) Group II is compared with Group I. Groups III-VI were compared with Group II. \( *P<0.05, **P<0.01, ***P<0.001. \) \( V. \) \( vinifera \): \textit{Vitis vinifera}
CONCLUSION

On the 28 days of oral administration of herbal *V. vinifera* restored body biochemical and histopathological alterations are due to the existence of different constituents. Higher activity was elicited at 400 mg/kg. Therefore, *V. vinifera* is regarded as an effective plant for treating obesity.

ACKNOWLEDGMENTS

The authors would like to thank Brilliant College of Pharmacy, for providing necessary facilities in the successful completion of the work.

REFERENCES