α-Glucosidase and Pancreatic Lipase Inhibitory Activity of SALCITAL-Plus – A Standardized Extract of Salacia reticulata

HEBBANI NAGARAJAPPA SHIVAPRASAD
Research & Development Centre, Olive Lifesciences Pvt. Ltd. KIADB
Industrial Area, Anthrasanahalli, Tumkur – 572106
Karnataka, India

BHANUMATHY MARIYANNA
Research & Development Centre, Olive Lifesciences Pvt. Ltd. KIADB
Industrial Area, Anthrasanahalli, Tumkur – 572106
Karnataka, India

DIVANJI MANOHAR
Research & Development Centre, Olive Lifesciences Pvt. Ltd. KIADB
Industrial Area, Anthrasanahalli, Tumkur – 572106
Karnataka, India

SUBRATA PANDIT*
Research & Development Centre, Olive Lifesciences Pvt. Ltd. KIADB
Industrial Area, Anthrasanahalli, Tumkur – 572106
Karnataka, India

SHARIQ AFSAR THANDU
Research & Development Centre, Olive Lifesciences Pvt. Ltd. KIADB
Industrial Area, Anthrasanahalli, Tumkur – 572106
Karnataka, India

VISHAL JAIN
Research & Development Centre, Olive Lifesciences Pvt. Ltd. KIADB
Industrial Area, Anthrasanahalli, Tumkur – 572106
Karnataka, India

PAVAN KUMAR B
Radiant Research Service Pvt. Ltd., Srinagar, B.S.K., 1st Stayl Banashankari,
Bangalore - 560056, India

Abstract:
Salacia reticulata has been used in Ayurvedic medicine for

* Corresponding author: subratapandit@olivelifesciences.com
diabetes and obesity since antiquity. SALCITAL™ Plus (SALCITAL-Plus) is a standardized extract of Salacia reticulata roots enriched with polyphenolic compounds including mangiferin. Combined study on α-glucosidase and pancreatic lipase inhibition is considered to be an ideal therapy for management of hyperglycemia and hyperlipidemia. In the present investigation α-glucosidase and pancreatic lipase inhibition potential of SALCITAL-Plus was evaluated and total polyphenol content was estimated. In order to standardize SALCITAL-Plus, reverse phase-high performance liquid chromatographic (RP-HPLC) method was developed to determine the mangiferin content. Results showed that SALCITAL-Plus has strong inhibition potential (IC₅₀ = 30.43±6.66 μg/ml) against α-glucosidase. Further the extract showed moderate inhibition potential against pancreatic lipase with an IC₅₀ value of 1.32±0.12 mg/ml. Results indicated that SALCITAL-Plus seems to be more inhibition potential compared to standard mangiferin. Greater inhibition potential of S. reticulata than standard mangiferin may be due to the synergistic activity of the other bioactive constituents present in the extract. Total polyphenols and mangiferin content in SALCITAL-Plus was found to be 14.10±1.53 g of gallic acid equivalents (GAE)/100 g of extract and 1.05% (W/W), respectively. The high polyphenol content of the extract may attribute to this inhibitory activity. These findings indicated that SALCITAL-Plus may be useful for the control of blood glucose and lipid concentration in hyperglycemia and hyperlipidemia. It can be recommended for weight management in obesity individuals.

Key words: Salacia reticulata, SALCITAL-Plus, mangiferin, standardization, polyphenol, α-glucosidase, pancreatic lipase

Introduction

Salacia reticulata (Family: Hippocrateaceae) is a large woody climbing plant, widely distributed in India, Sri Lanka, China, Thailand, Indonesia and Brazil. In Ayurvedic medicine this plant is being used from thousands of years as a specific remedy for diabetes, obesity as well as treatment of rheumatism, gonorrhea and skin diseases (Muraoka et al. 2010). The major phytoconstituents of S. reticulata include polyphenols such as mangiferin and salacinol; triterpenes such as kotalanol have
been proved for the anti-diabetic principles (Choudhary and Vijay Kanth 2005; Akase et al. 2011). Other phyto-constituents such as 1,3-diketones, dulcitol, leucopelargonidin, epi-catechin, phlobatannin, hydroxyferruginol, lambertic acid, kotalagenin 16-acetate, 26-hydroxy-1,3-friedelanedione, maytenfolic acid have also been reported from *S. reticulata* (Arunakumara and Subasinghe 2010). Leaves extract of *S. reticulata* has been reported to prevent human postprandial hyperglycemia (Shimoda et al. 1998) and to decrease the fasting plasma glucose level, body mass index (BMI) in mild type II diabetic patients (Kajimoto et al. 2000). Yoshikawa et al. (2002) reported that *S. reticulata* extract suppresses postprandial hyperglycemia and is being consumed as a food supplement in Japan for treatment of obesity and diabetes.

Universal advantages of herbal medicines are efficacy, safety, affordability and acceptability. Active principle always cannot be consistently quantified in a complex plant extract and the interpretation of assay data must be critically addressed using multiple strategies. With the growing interest in research on plant extract, there is also a growing concern regarding the action of bioactive phyto-compounds. Development of a marker profile and standardization is important to maintain quality control as well as to get the optimal concentrations of active constituents present in the plant extract (Pandit et al. 2011; 2012).

*S. reticulata* has now become a subject of broad studies for diabetes management, leads to increase in its consumption throughout the globe (Arunakumara and Subasinghe, 2010). α-Glucosidase and pancreatic lipase inhibitory activity of leaves extract of *S. reticulata* have been reported (Yoshino et al. 2009; Koga et al. 2013). α-Glucosidase activity of mangiferin has been studied and found to be more effective than standard acarbose (Dineshkumar, et al. 2010). There is no previous report on combined α-glucosidase and pancreatic lipase inhibitory activity
of standardized S. reticulata extract and mangiferin. Based on the above context, present study was designed to evaluate the \( \alpha \)-glucosidase and pancreatic lipase inhibition potential of SALCITAL™ Plus (SALCITAL-Plus) – A standardized extract of S. reticulata roots enriched with polyphenols including mangiferin. Further, total polyphenol content was estimated through Folin-Ciocalteu method. In addition, a reverse phase high-performance liquid chromatographic (RP-HPLC) method was developed to quantify mangiferin in SALCITAL-Plus.

Material and Methods

Chemicals and reagents

HPLC grade anhydrous potassium dihydrogen orthophosphate (KH\(_2\)PO\(_4\)), di-potassium hydrogen phosphate (K\(_2\)HPO\(_4\)), orthophosphoric acid (H\(_3\)PO\(_4\)), water, sodium carbonate (Na\(_2\)CO\(_3\)) and other analytical grade solvents were procured from Merck (Mumbai, India). 0.45 μm membrane filter were obtained from Millipore (Billerica, MA). Standard mangiferin, \( \alpha \)-glucosidase (Saccharomyces cerevisiae), 4-nitrophenyl-\( \alpha \)-D-glucopyranoside (PNPG), Whatman No. 1 filter paper and acarbose were procured from Sigma (Steinheim, Germany) used for \( \alpha \)-glucosidase activity study. Lipase from porcine pancreas, p-nitrophenyl butyrate (PNPB), orlistat, tris-HCl, acetonitrile, DMSO, EDTA and calcium chloride (CaCl\(_2\)) were purchased from Sigma (Steinheim, Germany), used for pancreatic lipase inhibition assay. 96 microwell plates were obtained from NUNC (Roskilde, Denmark).

Test materials

Roots of S. reticulata were purchased from local vendor and authenticated. SALCITAL-Plus was developed at Research and
Hebbani Nagarajappa Shivaprasad, Bhanumathy Mariyanna, Divanji Manohar, Subrata Pandit, Shariq Afsar Thandu, Vishal Jain, Pavan Kumar B - α-Glucosidase and Pancreatic Lipase Inhibitory Activity of SALCITAL-Plus – A Standardized Extract of Salacia reticulata

Development Center, Olive Lifesciences Pvt. Ltd. Tumkur, Karnataka, India.

α-Glucosidase inhibition assay

α-Glucosidase inhibitory activity of SALCITAL-Plus was evaluated based on chromogenic method described by Moradi-Afrapoli et al. (2012), with few modifications. In brief, 10 μl of test solution (10 – 80 μg/ml concentrations range) was mixed with 50 μl of phosphate buffer (0.1 M, pH 6.9) in 96 microwell plates. 25 μl α-glucosidase solution (1.25 U/ml) was added to this mixture and incubated at 37ºC for 15 min. Reaction was initiated with the addition of 25 μl PNPG (0.5 mM), as a substrate and incubated at 37ºC for 15 min. The reaction was terminated by addition of 100 μl Na₂CO₃ solution (0.2 mM). Absorbance of the wells was measured at 410 nm using microplate reader (BioTek, Winooski, USA) with integrated Gen5 software. Acarbose was used as a positive inhibitor of α-glucosidase. Same reaction mixture replacing test substance by buffer was used as control. Blank was prepared with the same reaction mixture replacing α-glucosidase and PNPG by buffer. All the experiment was carried out in triplicate. The percentage inhibition was determined by the following formula.

\[
\text{Percentage inhibition} = \left[1 - \frac{\text{sample} - \text{blank}}{\text{control}}\right] \times 100
\]

Pancreatic lipase inhibition assay

Pancreatic lipase inhibition potential of SALCITAL-Plus was determined by modified method of Bustanji et al. 2010. Crude porcine pancreatic lipase type II was prepared in tris-HCl buffer (pH 7.4). Different concentrations of test substances ranging from 125 to 2000 μg/ml were optimized to determine the IC₅₀ value. 100 μl enzyme solution was incubated with the
test substances for 1 min at 37°C. Volume was adjusted to 1 ml using the tris-HCl buffer. Absorbance was measured at 410 nm at 5 time points (1 - 5 min). The reaction was initiated by addition of PNPB (100 M) as substrate. Release of p-nitrophenol was measured at 410 nm, by UV spectrophotometer (Systronics, Mumbai, India). Blank contained the same mixture with denatured enzyme instead of pancreatic lipase type II. Enzyme activity was determined as an increase of the absorbance per minute. Rate of p-nitrophenol release can be calculated from the slope of the linear segment of absorbance versus time profiles. All the experiments were performed in triplicate. Orlistat was used as a positive control. Percentage inhibition was calculated based on the following formula. Percentage inhibition = [(Blank-Test)/Blank] X 100

**Standardization of SALCITAL-Plus by RP-HPLC**

SALCITAL-Plus was standardized by RP-HPLC using mangiferin as a marker compound. The RP-HPLC system (Shimadzu, Kyoto, Japan) consisting of two LC – 20AP controller pump; SPD-M20A PDA detector; SIL – 10AP autosampler with 20 μl loop and integrated LC solution software. Separation was achieved using Phenomenex ODS2 (250×4.6 mm; 5 μm) column (USA). The analytical parameters were optimized after screening a number of solvent systems and gradient elution. Separation was achieved with a two pump gradient program for pump A [0.136 g of KH$_2$PO$_4$ and 0.5 ml of H$_3$PO$_4$, volume make up to 1000 ml with water] and pump B [acetonitrile] as follows: initially 5% B, then increased gradually to 80% B until 28.0 min, again decrease gradually up to 5% B till 45 min, with continuous flow rate of 1.5 ml/min. Ideal resolution of chromatogram was achieved at 254 nm. Marker compound and *S. reticulata* extract were prepared in 70% alcohol and filtered through Whatman NYL 0.45 μm
syringe filter. The presence of mangiferin in extract was identified by comparing chromatographic peak with the retention time (RT) of the standard. The percentage of mangiferin in SALCITAL-Plus was determined by means of a calibration curve.

**Determination of total polyphenol content (TPC)**

TPC was determined spectrophotometrically according to the method described by Anesini et al. 2008, using gallic acid as a reference standard. In brief, 150 mg of *S. reticulata* extract was dissolved in 25 ml of 40% ethanol (V/V). Test solution was filtered through Whatman filter paper. 1.0 ml of the sample was transferred in duplicate to separate tubes containing 5.0 ml of 1/10 dilution of Folin-Ciocalteu’s reagent. 15.0 ml of sodium carbonate solution (20% W/V) was added to the reaction mixture. The tubes were then allowed to stand at room temperature for 120 min. Absorbance was measured at 750 nm against water. TPC in SALCITAL-Plus was derived from a standard curve of gallic acid ranging from 10 to 50 µg/ml concentration. The TPC was expressed as total gallic acid equivalent (GAE) in g/100 g of *S. reticulata* root extract.

**Statistical analysis**

Experimental data were expressed as mean±standard deviation (S.D.) for individual sample. The results were subjected to one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test by fixing the significance level at p<0.05 and above. The statistical analyses were performed using GraphPad Prism Version 5.0 (GraphPad Software, Inc., La Jolla, CA, USA).
Results and Discussion

Natural products can provide a vast pool of enzyme inhibitors with the development of potential clinical products. There is growing interest on α-glucosidase and pancreatic lipase dual inhibitors from medicinal plants with minimal side effects. This would provide an excellent new strategy for combating both diabetes and obesity (Bnouham et al. 2006; Mogale et al. 2011). Aim of the present work was evaluation of α-glucosidase and pancreatic lipase inhibitory activity of SALCITAL-Plus in order to determine blood glucose management and healthy lipid profile respectively and standardization of the extract.

Inhibition potential of SALCITAL-Plus on α-glucosidase

α-Glucosidase breaks down intestinal complex starches, dextrins, maltose and sucrose into simple monosaccharides. Inhibition of α-glucosidase results in delay of glucose absorption into the blood and suppresses hyperglycemia; consequently improve glycemic control (Heacock et al. 2005). In the present study SALCITAL-Plus and mangiferin showed good concentration dependent α-glucosidase inhibitory activity (Fig. 1).

(A)

![Graph showing inhibition of α-glucosidase](image)
S. reticulata extract and mangiferin showed highest inhibition potential of 80.18±12.02% and 56.94±6.35% respectively, at 80 μg/ml concentration. IC$_{50}$ values of the test substance against α-glucosidase have been represented in Table 1. S. reticulata extract showed lesser IC$_{50}$ value than mangiferin and acarbose. Results indicated that SALCITAL-Plus seems to be more potent α-glucosidase inhibitor compared to standard mangiferin. This may be due to synergistic activity of other bioactive constituents present in the extract. Study results strongly recommend hyperglycemic control of SALCITAL-Plus.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>IC$_{50}$ value (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SALCITAL-Plus</td>
<td>30.43±6.66**</td>
</tr>
<tr>
<td>Mangiferin</td>
<td>69.04±7.45n.s.</td>
</tr>
<tr>
<td>Acarbose</td>
<td>74.95±5.67</td>
</tr>
</tbody>
</table>

Table 1. IC$_{50}$ of SALCITAL-Plus on α-glucosidase
Each values represents the mean±S.D. (n=3). ** P<0.01 versus positive control acarbose; One-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test.

Presently marketed α-glucosidase inhibitors such as acarbose and miglitol are clinically effective for treatment of type 2 diabetes. These synthetic drugs produce adverse effects such as flatulence, abdominal pain, and diarrhea. These side effects
have directed researchers towards the discovery of safer natural blood glucose management therapy with minimum side effects (Li et al. 2008). Reports revealed that phytoconstituents could effectively inhibit α-glucosidase resulting decrease the absorption of carbohydrates from food (Wan et al. 2013). More interestingly mangiferin, kotalanol and salacinol of S. reticulata extract have been identified as antidiabetic principles through pharmacological studies (Arunakumara and Subasinghe 2010). Antidiabetic activity of mangiferin has also been reported by Dineshkumar et al. (2010). Strong α-glucosidase inhibitory effect of SALCITAL-Plus may be due to the high polyphenols content. Present investigation bare good aggregate of literature value and findings suggested that SALCITAL-Plus could decrease the blood glucose level by inhibiting the activity of α-glucosidase.

The inhibitory potency of SALCITAL-Plus on pancreatic lipase

Pancreatic lipase is the most important enzyme for the digestion of dietary fat. SALCITAL-Plus and standard mangiferin showed moderate inhibition potential on pancreatic lipase enzyme in dose-dependent manner (Fig 2).
Hebbani Nagarajappa Shivaprasad, Bhanumathy Mariyanna, Divanji Manohar, Subrata Pandit, Shariq Afsar Thandu, Vishal Jain, Pavan Kumar B - α-Glucosidase and Pancreatic Lipase Inhibitory Activity of SALCITAL-Plus – A Standardized Extract of Salacia reticulata

Fig. 2. Inhibition potential of orlistat (A), SALCITAL-Plus and mangiferin (B) on pancreatic lipase

Orlistat showed maximum percentage inhibition of 61.83±6.72 at 2 μg/ml concentration. SALCITAL-Plus produced maximum inhibition of 75.15±6.47% at 2 mg/ml concentration. SALCITAL-Plus showed lesser IC\textsubscript{50} value than mangiferin (Table 2). Greater inhibition potential of \textit{S. reticulata} extract than single compound mangiferin may be due to the synergistic activity of the other bioactive constituents present therein.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>IC\textsubscript{50} value (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SALCITAL-Plus</td>
<td>1.32±0.12***</td>
</tr>
<tr>
<td>Mangiferin</td>
<td>1.75±0.14***</td>
</tr>
<tr>
<td>Orlistat</td>
<td>0.001±0.00</td>
</tr>
</tbody>
</table>

Table 2. IC\textsubscript{50} of SALCITAL-Plus on pancreatic lipase
Each value represents the mean±S.D. (n=3). ***P<0.001 versus positive control orlistat; One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test.

Results indicated that SALCITAL-Plus was less potent to inhibit pancreatic lipase rather than orlistat. This lower potency compared to orlistat suggests that SALCITAL-Plus can be used as a complementary and alternative therapy in obesity control. \textit{S. reticulata} traditionally required several weeks to exert a noticeable hypolipidemic action to control obesity without any side effect. Moreover, safety profiles of \textit{S. reticulata} have been well established through subacute toxicity,
mutagenicity, antigenicity, phototoxicity study and quality control methods (Yoshikawa et al. 2002). Several reports suggested potential use of *S. reticulata* extracts in modification of the blood lipid profile and take part in obesity control (Hasani-Ranjbar et al. 2009; Harach et al. 2010; Shivaprasad et al. 2013). Study results revealed moderate pancreatic lipase inhibition potential of SALCITAL-Plus, demonstrated its hypolipidemic activity.

**RP-HPLC standardization of SALCITAL-Plus**

Standardization parameters and marker profile is crucial for quality control and consistent efficacy of medicinal plant products such as standardized extracts, herbal formulations, food supplements etc. and to get knowledge about the bioactive constituents present therein (Pandit et al. 2011a; 2011b). Quantity of mangiferin in SALCITAL-Plus was determined by RP-HPLC, using the external standard calibration technique under gradient elution, with a run time of 45 min. Mangiferin in *S. reticulata* extract was identified by comparing the respective RT. The RT was found to be 9.18 min without any interference. For constraining calibration curve, peak areas were plotted against the concentration of standards by means of linear regression. Five different concentrations of standard mangiferin, ranges from 200–1000 μg/ml showed good linearity. Good correlation was found between concentrations and peak area, with a correlation coefficient of more than 0.99. RP-HPLC chromatograms of SALCITAL-Plus and mangiferin have been represented in Fig. 3. Result was considered satisfactory and acceptable for subsequent quantitative analysis. Mangiferin content in the SALCITAL-Plus was found to be 1.05 % (W/W).
Polyphenolic constituents of *S. reticulata* extract exhibit lipolytic action by inhibiting enzymes involved in lipid metabolism, such as pancreatic lipase, lipoprotein lipase and glucose-6-phosphate ester dehydrogenase (Akase et al. 2011). Polyphenols in the plant extract has been reported as the main source of α-glucosidase and pancreatic lipase inhibitors (You et al. 2012; Mukherjee and sengupta 2013). In the present investigation TPC was found to be 14.10±1.53 g GAE/100 g of SALCITAL-Plus.

This is the first collective report on α-glucosidase and pancreatic lipase inhibitory activity of SALCITAL-Plus and its potent bioactive compound mangiferin. Published research suggests that there is a direct relationship between phenolic compounds from plant extract has inhibitory effect on α-glucosidase (Hanamura et al. 2006; Ikarashi et al. 2011). It also proved that polyphenol have inhibitory effects on lipid absorption and obesity through inhibition of pancreatic lipase (Adisakwattana and Chanathong 2011; Yoshikawa et al. 2002). Moreover, most phenolic phytochemicals reported to inhibit α-glucosidase enzymes in a non-competitive manner (Mogale, et al. 2011). The mechanisms of inhibition of α-glucosidase and...
pancreatic lipase was not investigated in detail, hence are not relevant to the aim of the study. It can be hypothesized that various group of polyphenols like catechins, flavonoids and tannins in SALCITAL-Plus play an important role for inhibition of α-glucosidase and pancreatic lipase. In addition, it can be predict that daily intake of this formulation may beneficial in managing hyperlipidaemia, reduce the risk of an individual in developing micro- and macrovascular complications including coronary heart disease, cardiovascular and cerebrovascular diseases. Further in-vivo studies on animal model must be conducted in order to confirm this assumption. Present studies demonstrated an appreciable antidiabetic and anti-obesity activity of SALCITAL-Plus by inhibiting α-glucosidase and pancreatic lipase enzymes.

Conclusion

The present study suggested that SALCITAL-Plus, a combination of α-glucosidase and pancreatic lipase inhibitor proved to be a valuable therapy for management of healthy lipid profile, weight managmente and blood glucose levels. High polyphenol content of SALCITAL-Plus inhibit glucose uptake, inhibit the rise in plasma glucose concentrations and delaying dietary fat digestion. It can be concluded that SALCITAL-Plus will be beneficial in diabetes and obesity individuals for maintaining normal blood glucose level and healthy lipid profile. Certain major factors such as mechanism of enzyme inhibition, in-vivo experiment followed by clinical study etc. need to be addressed further.

Acknowledgment

The authors are grateful to the Olive Lifesciences Pvt. Ltd., #30, Pride Quadra, 4th Floor, Hebbal, Bangalore- 560 024, India; for providing
Hebbani Nagarajappa Shivaprasad, Bhanumathy Mariyanna, Divanji Manohar, Subrata Pandit, Shariq Afsar Thandu, Vishal Jain, Pavan Kumar B - *a-Glucosidase and Pancreatic Lipase Inhibitory Activity of SALCITAL-Plus – A Standardized Extract of Salacia reticulata*

financial support for this work.

**BIBLIOGRAPHY:**


Bustanji, Yasser, Ala Issa, Mohammad Mohammad, Mohammad Hudaib, Khalid Tawah, Hatim Alkhatib,


Hebbani Nagarajappa Shivaprasad, Bhanumathy Mariyanna, Divanji Manohar, Subrata Pandit, Shariq Afsar Thandu, Vishal Jain, Pavan Kumar B - \textit{α-Glucosidase and Pancreatic Lipase Inhibitory Activity of SALCITAL-Plus – A Standardized Extract of Salacia reticulata}

Li, Yuhao, Tom Hsun-Wei Huang, and Johji Yamahara. 2008. “Salacia root, a unique Ayurvedic medicine, meets multiple targets in diabetes and obesity.” \textit{Life Sciences} 82: 1045–1049.


medicinal plants known to contain intestinal glucosidase inhibitors also inhibit pancreatic lipase activity – An ideal situation for obesity control by herbal drugs.”


Shimoda, Hiroshi, Shusuke Kawamori, and Yuzou Kawahara. 1998. “Effects of an aqueous extract of Salacia reticulata, a useful plant in Sri Lanka, on postprandial


